Frontiers in Potassium Nutrition:

New Perspectives on the Effects of Potassium on Physiology of Plants

Edited by: D.M. Oosterhuis and G.A. Berkowitz

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Foreword

FRONTIERS IN POTASSIUM NUTRITION:

New Perspectives on the Effects of Potassium on Crop Plant Physiology

A publication which includes the Proceedings from the C-2 Symposium on Potassium Nutrition in Plants

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The objective of this book is to integrate information about potassium (K) effects on cell physiology and metabolism and molecular aspects of K transport across membranes, with an understanding of how the nutrition of this major plant cation affects crop plant growth, development and performance. The publication is presented in two complementary sections. The first section covers basic perspectives focusing on how K movement across plant cell membranes and within the plant is both facilitated and regulated and also how K status interacts with basic aspects of cell function. This basic information regarding K transport and interaction with physiological function will provide a background context for the following section, which focuses on developing a new and fuller understanding of how K nutrition and cultural management interact with crop plant performance and quality factors. This integration of basic and applied aspects of K nutrition will incorporate work done on a range of plants, including arabadopsis, maize, wheat, cotton, citrus, and alfalfa.

The rationale underlying this proceedings is based on several factors. Widespread occurrences of K deficiency during recent years have been observed in several major crop plants grown in the U.S. Although K plays a vital role in numerous plant functions, our understanding of the physiology of K nutrition and how it impacts growth and development of any one crop plant is far from clear. Recent advances in our understanding of the molecular nature of plant K transport proteins and K interaction with metabolic function provide an excellent context for a new evaluation of how management of K nutrition in crop plants can impact growth and yield. Finally, new technologies have emerged which allow for the molecular/genetic manipulation of crop plants. Thus, new understanding of the underlying mechanisms of plant K nutrition can be applied to the engineering of crop plants for enhanced performance under a range of environmental conditions. The aim of the symposium was to bring together scientists working on different crops and, focusing on various aspects of K nutrition, to develop through the interactions facilitated by the symposium a better overall understanding of the role K has in plant growth and function.

The impetus for the proceedings was a "special symposium" at the American Society of Agronomy meetings in 1996. It was decided at that time that there was sufficient "new" information on K covering numerous basic and applied fields to warrant a book to bring this information together and make it easily available to all interested. Dr. Konrad Mengel from the Institute fur Pflanzenernahrung, Suedenlage, Germany, presented the opening talk, which provided an overview of the involvement of K in plant metabolism at the whole plant level. The development of a basic understanding of K involvement in cell and plant function was also facilitated during the first session by presentations on K involvement in protein and carbohydrate metabolism and the molecular, biophysical, and physiological aspects of K transport. Information on K ion channels was presented within the context of stomatal function, cytosolic homeostasis, regulation of K transport, and interaction of K uptake with salinity stress. Presentations by other prominent authorities covered topics such as K uptake by crop plants, K fertilizer management and root system function, plant/soil interactions, and the role of K in leaf gas exchange, fiber quality, and stomatal conductance of crops grown under water limitation. A final chapter was provided on agronomic management of K in the twenty-first century to provide an overview of where we are and what is in store in the near future. The information presented in this proceedings should facilitate the development of new goals for basic studies aimed at expanding our understanding of plant physiology and the application of such information to the improved cultural management of crop plants. The cloning of genes encoding K transport proteins and the characterization of the function of these transporters have identified specific targets for genetic engineering of crop plants for enhanced performance under environmental stress. It is our belief that the publication of a compendium of information related to such a significant topic of plant physiology and crop management will be of immense benefit to those scientists working in this research area, as well as to students and others who are new to the field.

Derrick Oosterhuis and Gerald Berkowitz (editors)

Acknowledgments

The editors wish to express their appreciation to all those individuals who helped with the arrangements for the symposium which provided the basis for this book. In particular we wish to thank Mr. Keith Schlesinger of the American Society of Agronomy and Dr. B.C. Darst of the Potash & Phosphate Institute for providing funds for the invited speakers from overseas. We also extend our gratitude to Ms. Marci Milus for help in typing and preparing this publication for the publisher.

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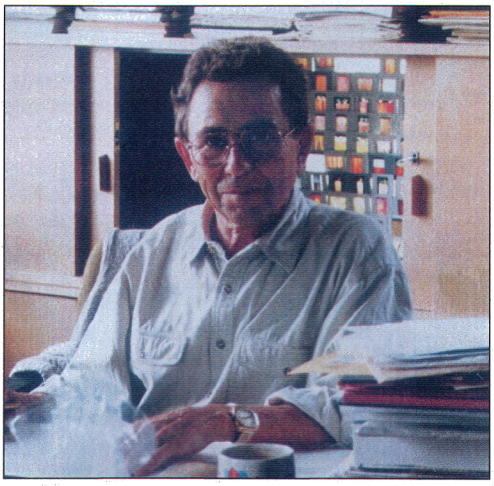

In memory of Horst Marschner, 1929-1996

Horst Marschner died September 23, 1996 after a very short illness from malaria, which he probably contracted when visiting research projects in West Africa over the summer. Dr. Marschner was one of the foremost experts in plant mineral nutrition. His book, *Mineral Nutrition of Higher Plants* (second edition, 1995 from Academic Press, London) is used as a textbook in many universities around the world. All who worked with him knew him as a stimulating and generous teacher, mentor, and colleague.

Dr. Marschner was born in 1929 in Zuckmantel, now in the Czech Republic. After the end of the war, the family moved to Thüringia, which became part of the German Democratic Republic. He worked as a farmer and went to an agricultural school before he started to study agriculture and chemistry at the University of Jena. He earned a Ph.D. in agricultural chemistry in 1957 and then joined the Institute for Research on Cultivated Plants in Gatersleben. During these years, he developed not only his interest in modern techniques for studying plant nutrient uptake but also a distrust of mixing dogmatic political interests with scientific research. Thus, in 1960, he and his wife decided to go to West Germany, took a train to Berlin, and joined the research group at Stuttgart-Hohenheim. In 1966, he become full professor of plant nutrition at the Berlin Technical University and, since 1977, was director of the Institute of Plant Nutrition at the University of Hohenheim.

Despite the success of his professional career and his heavy workload, he maintained an interest in new developments in agronomy, botany, and soil science. Personally modest, his office door was always open to students or visitors, and he tried always to understand and learn from the people he met. His reputation as a referee for scientific journals, as well as for funding agencies or government bodies, was particularly high because of his wide knowledge and experience and his interest in the progress of science without personal bias.

Dr. Marschner enjoyed discussing ideas with his co-workers, both students and established scientists. He combined a sharp mind with a simple but persuasive way of presenting his research findings. At the beginning of his career, he mainly studied the uptake of mineral nutrients, but then extended this to include nutrient transport and use within the plant. Starting in the 1970s, his research greatly advanced the understanding of rhizosphere processes and iron uptake by plants. Later, he also concentrated on environmental aspects of plant nutrition, e.g., the side effects of high rates of agricultural fertilizer use, heavy metal contamination of soils, and the effect of changes in forest ecosystems on the uptake and use of nutrients by trees. Dr. Marschner also published extensively on the adaptation mechanisms of plants to adverse soil conditions and low nutrient supply.

Chapter 1:
Integration of Functions and Involvement of Potassium Metabolism at the Whole Plant Level

Konrad Mengel

Institute of Plant Nutrition of the Justus Liebig University
Südanlage 6, D-35 390 Giessen, Germany

Introduction

In recent years, substantial new research progress has provided further insights into the role of potassium (K) in plant metabolism, particularly in the area of membrane transport of K^+. It has been found that most K^+ fluxes are mediated by K^+ selective channels present in different tissues of plants, animals, and fungi[31]. Potassium ion channels are structurally the simplest in the superfamily of cation channels and are characterized by a short section in the pore region which is responsible for the K^+ selectivity[34]. Both outwardly and inwardly rectifying K^+ channels are present in which the K^+ flux follows the electrical potential gradient[13, 69]. A variety of different types of channels occurs, including Ca^{2+} or Na^+ activated K^+ channels, K^+ channels which open due to membrane hyperpolarization [voltage-gated channels[12]] and others controlled by cyclic nucleotides. Living cells, including those in plant tissues, have numerous mechanisms for controlling influx and efflux of K^+ across biological membranes[26]. The need for this diversity of K^+ channels is not yet completely understood, but it is evident that K^+ is very mobile in all biological tissues.

Potassium ion fluxes across membranes are frequently associated with ion pumps. In this context, proton pumps such as the plasmalemma H^+ ATPase[81], the H^+ ATPase in chloroplasts[22] and the H^+ ATPase and the H^+ pyrophosphatase of the tonoplast[70] are of utmost importance. The action of these pumps results in the hyperpolarization of membranes, which drives the passive uptake of K^+ through rectifying K^+ channels[41]. Potassium ion uptake is not only brought about by the passive influx of K^+ through channels. Recent experiments have shown that uptake of K^+ may occur against the electrochemical gradient of K^+ across the plasmalemma in the form of a H^+/K^+ cotransport[74, 88]; even a Na^+/K^+ cotransport is proposed as an active mechanism of K^+ uptake[71].

Most of the work mentioned above was carried out with plant parts, plant organelles, and with isolated membranes. Experimental work carried out using whole plants has not received much attention in the last decade. For a complete understanding, however, the complex mechanism of crop growth and the synthesis of important molecules in relation to K^+ nutrition in the whole plant must be considered. For crop production, it is of special interest to know which process directly or indirectly involved in growth and synthesis is the most sensitive to K^+ supply.

Certainly the studies cited above document remarkable progress in understanding the role of K^+ in plant metabolism. This new knowledge contributes to a better understanding of the functions of K^+ in crop production as outlined below.

Potassium Uptake and K^+ Long-Distance Transport

When young intact corn (*Zea mays*) plants are placed with their roots in distilled water, protons are released from the roots into the outer medium and, over a period of several hours, the initial pH of the water is depressed from 6.0 to 4.8 (**Figure 1**). On replacement of this pH-depressed water by distilled water at pH 6.0, a similar pH depression again takes place. This process can be repeated several times, showing the same pattern of pH depression as indicated by the fluctuating line in **Figure 1**. The lower line in **Figure 1** shows the treatment in which the water of the root medium was not replaced; the upper line shows the control (water without plants)[59]. Addition of vanadate retarded, whereas fusicoccin promoted, the proton release. At low temperature, no proton release was observed. From these results it was concluded that plasmalemma H^+ ATPase activity does not require cations in the outer solution, and that its pumping mechanism is strong enough to overcome the H^+ buffering capacity of the root apoplast.

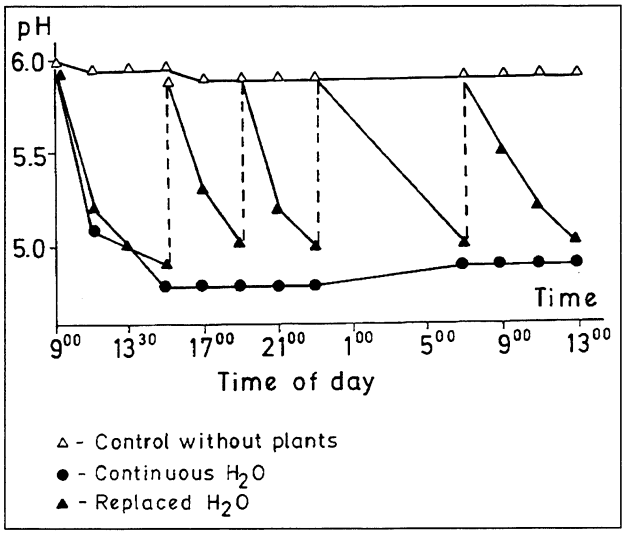

Figure 1. **Proton excretion of roots from young intact maize plants into distilled water. The change of pH is reflective of H^+ release by roots[58].**

If, however, the outer solution contains cations, H^+ release rates are higher for the cation species applied according to the following sequence $Na^+ < Ca^{2+} < Mg^{2+} < K^+$, the highest rate being obtained with K^+. The H^+ release rate in the presence of external K^+ is about four times higher than into distilled water. Transferring plants from a nutrient solution into distilled water resulted in an immediate electrical potential shift from -120 to -190 mV. Presumably, at this hyperpolarization of the plasma membrane, the activity of the proton pump is restricted because a steeper electrochemical H^+ gradient has to be overcome in order to pump protons. It can be assumed that the uptake of cations drives the proton pump by depolarizing the plasma membrane.

This effect is particularly true for K^+, which is taken up at the highest rate. The K^+ is thought to have an additional beneficial effect on the ATPase activity[9].

Potassium ion uptake likely occurs via passive influx through the plasmalemma K^+ channels. This uptake mechanism is not a K^+/H^+ antiport as was formerly supposed[42]. However, it is the plasmalemma H^+ pump which initiates the K^+ uptake, and the K^+ taken up maintains the activity of the pump. The plasmalemma ATPase is of universal importance for the "biochemical pH stat" of the cytosol and for the uptake of numerous metabolites including plant nutrients such as nitrate and phosphate. For this reason it has been called the master enzyme by Serrano[81]. As discussed above, the intensity of H^+ pumping depends on the presence of cations, especially of K^+ in the outer solution. This positive K^+ effect may not only result from a depression of the electropotential gradient across the membrane but also from the cytosolic K^+ concentration. As reported by Briskin and Poole[9], K^+ promotes the dephosphorylation of the ATPase during the proton-pumping process.

From the relationship between the plasma membrane ATPase and passive K^+ influx through K^+ selective channels, a simple mechanism of K^+ uptake may be postulated. Different types of K^+ channels may allow only passive influx or efflux of K^+ according to the K^+ electrical potential difference across the membrane. There is experimental evidence from various plant tissues of the presence of two principal types of rectifying K^+ channels, one inwardly and one outwardly directed[77, 17, 8]. The outwardly directed channel is blocked by Ca^{2+} or Ba^{2+} [8]. This is an interesting finding which shows that K^+ efflux out of the cell may be blocked by high concentrations of Ca^{2+} in the outer solution. Although these results of Bouteau et al.[8] were obtained with protoplasts of lactifers isolated from *Hevea brasiliensis,* they may have a more general application to root cortical cells. These cells could lose K^+ under energy stress, affecting the negative cytosolic charge. If, however, Ca^{2+} were present in the outer solution in a concentration of about 5 mM, the outwardly-directed K^+ channels would be blocked and no major K^+ loss would occur. Since the soil solution Ca^{2+} concentration even in acid soils is in the order of 5 mM, major K^+ losses are minimized during a transient energy stress. This effect of Ca^{2+} is in good agreement with earlier results of Mengel and Helal[54] who found that with whole plants Ca^{2+} in the outer solution did not affect K^+ uptake by roots but blocked K^+ efflux. This phenomenon was known as the Viets effect in the older literature.

Figure 2 shows the results of experiments carried out with young intact maize plants exposed to a nutrient solution with normal nutrient concentrations except for K^+, the concentration of which was lowered to 25 mM. Plants were kept in this solution for four days; in one treatment with continuous light and in the other treatment in the dark[16]. During the first 8 h, in both treatments the K^+ concentration of the nutrient solution was lowered but after this period the plants kept in the dark began to release K^+ in a near linear fashion throughout the experimental period. The absolute loss of K^+ was low and the K^+ concentration of roots was hardly affected, presumably due to the Ca^{2+} concentration in the outer solution which was at 5 mM. Plants kept in the light lowered the K^+ concentration to a level of about 10 mM. It may be deduced that plants in the dark lacked the energy for retaining K^+ in the root cells. This view is in accord with the diurnal rhythm of K^+ uptake reported by Le Bot and Kirkby[39] and Macduff and Dhanoa[44]. Also, Schubert and Mengel[79] (with whole plants of *Zea mays),* and Mengel and Malissiovas[56] (with whole plants of *Vitis vinifera*) found that proton excretion by roots was higher in the light than in dark.

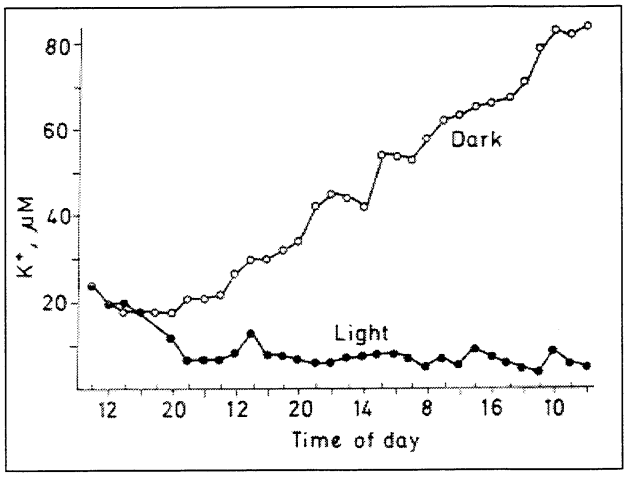

Figure 2. K^+ concentration changes in the nutrient solution with young intact maize plants with light and dark application over a period of four days[16].

The low K^+ concentration of about 10 mM in the nutrient solution shown in Figure 2 was lower by about a factor of 10^4 than the cytosolic K^+ concentration which is in the order of 100 mM. From this K^+ concentration difference it can be deduced, using the Nernst equation, that under equilibrium

conditions the electropotential difference between the outer solution and the cytosol should be about 230 mV. Such a difference is rated as very high for intact plants. It seems more likely that at this low K^+ concentration in the outer solution, an active K^+ uptake mechanism comes into play as recently described by Schachtman and Schroeder[74] as a K^+/H^+ cotransport or even a K^+/Na^+ cotransport as suggested by Rubio et al.[71]. The latter Na^+/K^+ cotransport, however, has been questioned by Walker et al.[88]. The K_m in wheat roots found by Schachtman and Schroeder[74] for the K^+/H^+ cotransporter was 50 mM K^+ which is relatively high when compared with the low K^+ concentration shown in **Figure 2**. One may speculate as to whether a K^+ transporter with an even higher affinity for K^+ may be present in corn roots. Such high affinity K^+ transporters may depress the K^+ concentration at microsites of root surfaces to very low concentrations, promoting K^+ release from K^+-bearing minerals[85]. Under such conditions interlayer K^+ of micas present in the silt fraction is strongly exploited by plants[58]. In the long run, this form of soil mineral K^+ mining leads to the destruction of soil minerals[87].

From the above considerations, it is evident that the plasma membrane of plants contains diverse K^+ uptake systems which may be grouped into two main categories, the channels allowing a passive flux and the transporters mediating an active uptake of K^+ from the nutrient solution into the cytosol. The channels may be directed inwardly and outwardly. They may be voltage-gated or activated by cations or cyclic nucleotides[31]. As yet, we do not know all the uptake systems for K^+ located in the plasma membrane of plants, nor do we know how they are regulated or the response of such systems to insufficient K^+ supply. It is known, however, that the internal K^+ status plays a decisive role in this regulation. At high intracellular K^+ levels, the K^+ uptake rates are low and *vice versa*[26]. It seems reasonable to suppose that with this broad spectrum of K^+ uptake systems, plants my well adapt to varying K^+ concentrations in the soil solution.

The tonoplast is a further membrane which must be traversed by K^+. According to Hedrich and Schroeder[31], the plasmalemma much resembles the plasma membrane of fungi and animals while the tonoplast is similar to the membrane of lysosomes. The vacuolar membrane, the tonoplast, contains two types of proton pumps, a H^+ ATPase and a pyrophosphatase[70]. The vacuolar H^+ ATPase differs from that of the plasmalemma in that it is not inhibited by vanadate but is inhibited by halides. The vacuolar pyrophosphatase is ubiquitous in the plant kingdom but otherwise only known in a few phototrophic bacteria. It is selectively stimulated by K^+ and requires Mg^{2+} presumably for allosteric modulation. The enzyme not only pumps H^+ but also facilitates active K^+ transport, which is obviously of utmost importance for the transport of K^+ into the vacuole. Ion channels in the tonoplast are characterized by a low selectivity for monovalent cations[31]. The vacuole may contain K^+ concentrations in the range of 100 mM, so the pyrophosphatase pumping K^+ into the vacuole seems to be of high importance in that this active pumping mechanism may overcome the electrochemical equilibrium for K^+ between the cytosol and the vacuole. Since the negative charge in the vacuole is lower than in the cytosol, active K^+ uptake will frequently be necessary. It also has to be borne in mind that vacuolar K^+ is an important osmoticum. The stoichiometric relationships of the vacuolar pyrophosphatase have not yet been completely elucidated. There is evidence that per molecule pyrophosphate hydrolyzed, three monovalent cations can be translocated and that the complex has three negative binding sites for which H^+ and K^+ may compete[70]. Potassium ions in the vacuole also serve as a K^+ store and are accumulated under conditions of high K^+ supply and retranslocated into the cytosol if required[19, 40].

Long distance transport of K^+ in whole plants is brought about by movement in the xylem and phloem. Xylem flow itself is promoted by K^+. Baker and Weatherley[2] found that K^+ increased the exudation rate of *Ricinus communis*. A similar observation was made by Mengel and Pflüger[57] with *Zea mays*. It is supposed that K^+ released into xylem vessels depresses their water potential and thus induces water flow into the xylem sap. This positive K^+ effect is not only true for water transport, but also for the translocation of organic and inorganic solutes in the xylem sap[60]. The K^+ concentration in the xylem sap is in a range of 10 to 20 mM[36] and 60 to 110 mM in the phloem sap[30], which means that K^+ is translocated with high rates in vascular tissues to various plant parts and organs. From this, it follows that the K^+ translocated *via* the xylem sap into upper plant parts is partially recycled in the phloem sap to the roots[46]. An important function of K^+ in the xylem and phloem sap is to balance negative charges, in the xylem sap mainly nitrate, in the phloem sap mainly organic anions[46].

Physiological Source

Photosynthesis is the most important physiological mechanism in green plants. This process involves the transfer of solar energy into chemical energy with ATP and NADPH being the first forms of chemical energy produced in the photosynthetic process. The synthesis of these two coenzymes is positively influenced by K^+ as is shown in **Figure 3** from early work of Pflüger and Mengel[66]. The experiments were carried out with chloroplast suspensions from plants well supplied and less well supplied with K^+. From the lower part of **Figure 3** showing photophosphorylation (ATP synthesis), it is evident that the rate of photophosphorylation was higher in the chloroplasts with the higher K^+ supply. Increasing K^+ concentration in the suspension as depicted on the x-axis of **Figure 3**, however, had no impact on ATP synthesis. The upper

part of **Figure 3** shows the rate of photoreduction as measured by the reduction of ferricyanide. A considerable increase in photoreduction occurred as the result of the K^+ status of the chloroplasts and also from the K^+ additions to the suspension. The experiments were carried out with *Helianthus annuus, Spinacia oleracea*, and *Vicia faba*, the results of the latter being shown in **Figure 3**. With all three species, the same positive impact of K^+ on photosynthetic activity was observed. More recent experimental results are helpful in interpreting the findings cited above. Berkowitz and Peters[6] found an ATPase located in the inner envelope of chloroplasts pumping protons out of the stroma and inducing a K^+ flux into the stroma through K^+ selective channels. The importance of K^+ for the proton pumping by the envelope-located ATPase was shown by Shingles and McCarty[82] who found that in the treatment with no K^+, the activity of the ATPase was very low while in the treatment with K^+, the activity measured by the hydrolyzed phosphate was increased by a factor of 30. According to Berkowitz and Peters[6], were it not for a system to pump protons out of the illuminated chloroplast, the increase in stromal pH developed as a consequence of H^+ pumping into the thylakoid lumen would quickly dissipate. This high pH is a prerequisite for an efficient transfer of light energy into chemical energy which was evidenced by the higher rate of O_2 produced by photolysis in the treatment

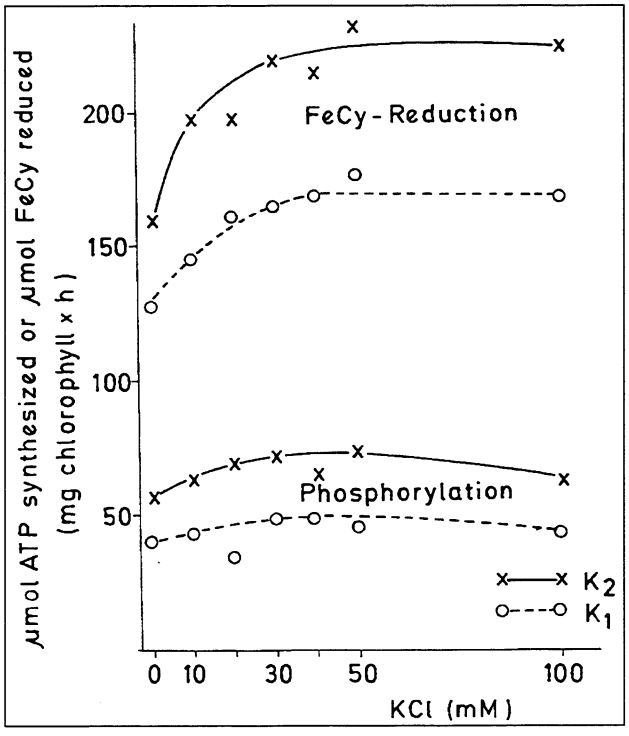

Figure 3. **Photoreduction (upper part) and photophosphorylation (lower part) of a suspension of broken chloroplasts obtained from *Vicia faba* at varying assay suspension K^+ concentrations. Plants were cultivated with a high (K_2, 3.0 mM) or low (K_1, 0.1 mM) growth medium K^+ concentration[65].**

with an elevated K^+ concentration[90]. The finding confirms the results of Pflüger and Mengel[66] shown in **Figure 3**. The favorable effect of K^+ on the CO_2 assimilation of wheat was shown by Pier and Berkowitz[67]. The influence of K^+ was particularly pronounced under conditions of an insufficient water supply, an observation which is of practical relevance.

The positive impact of plant K^+ status on CO_2 assimilation and energy turnover is shown in **Table 1** from the work of Peoples and Koch[64]. Low K^+ concentrations in alfalfa leaves were associated with low CO_2 assimilation rates and high mitochondrial respiration. These results demonstrate that under the condition of a poor K^+ status, leaf metabolism requires an additional ATP supply from respiration and thus draws from stored energy in the tissue. Increasing leaf K^+ concentrations also promoted photorespiration, but its absolute contribution to the energy status of leaves was low as compared with the CO_2 assimilation rates. From the interesting results of Peoples and Koch[64], the general conclusion may be drawn that the K^+ status of leaves is of fundamental importance for the energy status of the whole plant because high CO_2 assimilation rate leads to high rates of organic carbon fluxes from the leaves into other plant parts, particularly into storage organs and into meristematic sinks. As will be shown below, this effect is of paramount importance for crop growth and production.

Table 1. **Effect of K^+ on CO_2 assimilation, photorespiration and mitochondrial respiration as related to K^+ concentration in alfalfa leaves (after 64).**

mg K/ g DM	CO_2 assimilation	Mitochondrial respiration	Photo-respiration
	------- mg/dm²/h -------		dpm/dm²
12.8	11.9	7.6	4.0
19.8	21.7	3.3	5.9
38.4	34.0	3.1	9.0

Phloem Transport

In the older literature, numerous researchers reported that K^+ promotes phloem transport[55]. The question as to whether this is an indirect effect originating from the beneficial influence of K^+ on photosynthesis was treated by Mengel and Viro[62]. The main results of this investigation are shown in **Table 2**. Potassium nutrition had no influence on photosynthesis but the distribution of labeled photosynthate was clearly influenced by K^+. The proportion of labeled material in tomato fruits is much higher in the treatment with the higher K^+ supply, suggesting that phloem transport responds more sensitively to K^+ supply than does photosynthesis. Investigations of phloem sap collected from *Ricinus communis* supplied in one treatment with 0.4 mM K^+ and in one treatment with 1.0 mM K^+ yielded the results shown in **Table 3** from the work of Mengel and Haeder[52]. Concentrations of the ma-

jor solutes in the phloem sap were not influenced by K^+ with the exception of malate and K^+. The solute potential was significantly higher, and the phloem flow rate in the treatment with high K^+ supply was almost twice as high as compared with the low K^+ supply. Consequently, long distance transport of photosynthates was considerably better in the plant with the higher K^+ status. The higher solute potential in these plants indicates that sieve tubes were better able to attract water and thus improve the "push" of the phloem flow according to the "push and pull" concept of Geige[24]. Geiger and Conti[14] did not find a positive influence of K^+ on the export of organic carbon from the leaves of *Beta vulgaris*. The lowest K^+ concentration used by these authors, however, was 2 mM, a high concentration which is still about 10 times higher than that generally found in the soil solution. In the work of Mengel and Haeder[52] with *Ricinus communis* and in the work of Mengel and Viro[62] with tomatoes, the lowest K^+ concentration was 0.4 mM and these plants did not show K^+ deficiency symptoms.

Table 2. Effect of K^+ supply on the distribution of ^{14}C and $^{14}CO_2$ assimilation in tomato plants. In treatments K_1 and K_2 plants were grown with 1 or 10 mM K^+ in the nutrient solution, respectively (after 61).

		K_1	K_2
		- - - - % distribution - - - -	
	dpm / plant	123×10^6	111×10^6
Plant part	dpm / FM	110×10^3	120×10^3
Leaves		52.7	49.6
Fruits		6.0	15.2
Stems		37.7	36.6
Roots		3.7	2.6

Table 3. Concentration of solutes in the phloem sap of *Ricinus communis* as related to K^+ supply. K_1 and K_2 = 0.4 and 1.0 mM K^+ in the nutrient solution, respectively (after 52).

	K_1	K_2
K^+, mM	47.00	66.00**
Sucrose, mM	228.00	238.00
Total Amino acids mM	192.00	192.00
Malate, mM	0.83	1.32*
Solute potential, MPa	- 1.25	- 1.45***

Flow rate in the K_2 treatment was almost twice as high as in the K_1 treatment.
*, **, *** significant difference at the 5%, 1%, and 0.1% level.

Why K^+ has this favorable effect on phloem transport is as yet not completely understood. The work of Mengel and Haeder[52] indicates that it is the process of phloem loading and not processes in the physiological sink which are directly influenced by K^+. Phloem loading of photosynthate is brought about by proton cotransport of sucrose and amino acids across the plasmalemma[11] of the companion sieve cell complex driven by the plasmalemma ATPase (**Figure 4**). As already discussed, K^+ transport across the membrane may decrease the electrical potential difference and thus reduce the energy demand required for the pumping of protons across the membrane. In addition, cytosolic K^+ promotes the ATPase by stimulating the dephosphorylation of phosphate bound to the catalytic site of the enzyme[9].

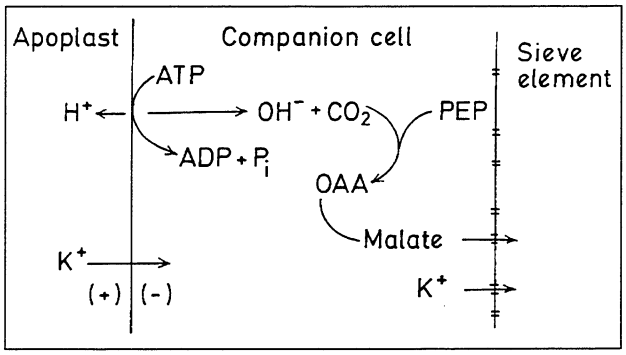

Figure 4. Scheme of ATPase activity in the plasmalemma of a companion cell driving K^+ uptake and thus inducing a surplus of OH^- in the cytosol which results in the synthesis of malate. This model portrays the sieve element loading K^+ and malate.

According to van Bel and van Erven[3], K^+ particularly promotes the uptake of sucrose and glutamine at higher pH in the apoplast. Such high pH levels may occur if many protons are recycled back *via* proton cotransport with photosynthate into sieve tubes. Therefore van Bel and van Erven[3] suppose that the form of cotransport is dependent on the apoplastic pH, with K^+ cotransport occurring at a relatively high pH and proton cotransport at low pH. In addition to the effect of K^+ on ATPase activity, K^+/assimilate cotransport across the plasmalemma of the companion cell could be the basis for the positive impact of K^+ on phloem loading. An indirect indication that a higher K^+ supply stimulates the plasmalemma ATPase of the sieve tube companion cell complex is the significantly increased malate concentration in the phloem sap (**Table 3**). Increased proton pumping out of the cytosol is associated with an equivalent increase of OH^- production in the cytosol, which for pH stat reasons must be neutralized by phosphoenolpyruvate (PEP) carboxylation with malate as an end product. The favorable influence of K^+ on phloem loading and phloem flow is of fundamental importance for supplying the physiological sink with organic carbon and organic nitrogen and, therefore, may have an impact on metabolic processes in physiological sinks as considered below.

Physiological Storage Sinks and Secondary Plant Metabolism

Table 4 shows the effect of K^+ nutrition on the incorporation of ^{15}N in wheat grain proteins. Incorporation rates were higher for all four protein fractions, although the K^+ concentration in the grain was virtually the same under both treatments. Potassium concentrations in the leaves, however, were 16 mg/g DM in the treatment with

0.3 mM K^+ in the nutrient solution and 34 mg/g DM in the treatment with 2 mM K^+ in the nutrient solution[61]. The improved protein synthesis as a result of the higher K^+ supply obviously was not because of a direct effect of K^+ on protein synthesis, but resulted from a higher import of photosynthate which leads to higher levels of amino acids and energy in the form of sucrose. This assertion is supported by the finding that grain of the high K^+ supply treatment not only showed a higher rate of grain protein synthesis, but also the concentrations of ^{15}N labeled and non labeled soluble amino acids were higher in the treatment with the better K^+ nutrition. Apart from this example, K^+ may also directly promote protein synthesis by enzyme activation as shown below. The positive effect of K^+ on grain filling and single grain weight[80] is in agreement with findings of Koch and Mengel[38], who reported that K^+ favored the translocation of assimilates to the ears of spring wheat and also had a positive effect on the import of organic carbon in wheat grains[51]. Analogous results have been reported for the import of organic carbon into potato tubers[28] and for the import of assimilates in root nodules of *Vicia faba* with a favorable impact on nodule growth[28] and N_2 fixation[53]. The oil concentration in rape seeds (*Brassica napus,* L. ssp. oleifera) was almost doubled with 1.4 mM K^+ in the nutrient solution as compared with 0.2 mM K^+[50]. Lipid and protein production in oat grains were favorably influenced by adequate K^+ nutrition[48]. Lipid production in particular requires much energy, and there are good reasons to assume that under low K^+ treatments the provision of developing seeds with organic carbon for oil production is insufficient.

Table 4. Effect of K^+ on the incorporation of ^{15}N into grain proteins of wheat and K concentration in grains. K_1 and K_2 = 0.3 and 1.0 mM K^+ in the nutrient solution, respectively (after 80).

	K_1	K_2
Albumin, mg ^{15}N / kg	42.4	67.0
Globulin, mg ^{15}N / kg	36.4	49.2
Prolamin, mg ^{15}N / kg	108.0	151.0
Glutelen, mg ^{15}N / kg	130.0	194.0
K-concentration mg K / DM	4.9	5.0

One may speculate that for the synthesis of secondary plant metabolites, the supply of energy is of importance and thus also the K^+ status of plants. As shown in **Table 5**, K fertilizer application had a marked impact on tomato yield and particularly on the concentration of vitamin C in fruits[1]. Tomato fruits suffering from K^+ deficiency may show "green back" which is a lesion in the tissue around the petiole and affects the fruit quality considerably[20]. According to Sun Xi and Rao Li-Hua (unpublished observations), the epicuticular wax of cotton leaves is poorly developed in plants suffering from K^+ deficiency. This wax affords a protection of leaves against water loss and pathogen attack. The positive effects of K^+ listed above are related both to the photosynthetic efficiency and the phloem transport promoted by K^+ and presumably not by direct enzyme activation by K^+.

Table 5. Effect of K fertilizer application on yield and quality of tomato fruits (after 1).

Treatment, kg K_2O / ha	Yield, t / ha	DM, %	Vitamin C, mg / kg
0	63.4	4.4	173
66	65.5	4.9	192
133	84.9	5.4	221
199	74.1	5.4	263
266	62.8	4.4	221

The situation may be different, however, in cases where sucrose is stored, e.g. in the storage tissue of sugar beets and sugar cane. Here the sucrose transport into the vacuole may be influenced by K^+ as shown in **Figure 5**. Saftner and Wyse[72] reported that sucrose is translocated across the tonoplast *via* K^+ sucrose cotransport coupled with a K^+/H^+ antiport. This action drives K^+ out of the vacuole back into the cytosol where a K^+ concentration of 100 to 120 mM is required for maximum sucrose import into the vacuole. Saftner et al.[73] reported that the sucrose concentration in cells of sugar beet storage roots was 62 mM in the apoplast, 76 mM in the cytosol, and 514 mM in the vacuole. Sucrose uptake by the cells was characterized by a biphasic pattern in relation to the sucrose concentration applied, displaying both a linear component and a saturable component. The latter was accounted for by passive influx of sucrose across the plasmalemma and the linear component by transport across the tonoplast. The fact that this sucrose uptake is linear over a sucrose concentration range from 0 to 300 mM indicates that a very efficient sucrose uptake system exists which is likely to be a K^+/sucrose cotransport system driven by a H^+/K^+ antiport. This sucrose uptake mechanism contrasts with the concept of Briskin et al.[10] for sugar beet roots. They postulate a sucrose/proton antiport in which the H^+ gradient between the vacuole and cytosol drives sucrose import, and the regeneration of the gradient is brought about by tonoplast proton pumps. Getz et al.[25], investigating sucrose uptake by tonoplast vesicles isolated from sugar cane storage tissue, also postulated a sucrose/ proton antiport. The problem of sucrose import into the vacuole is not only of importance for sucrose but also for fructan storage tissues[68], since fructan storage depends also on the import of sucrose into vacuoles. In grasses, fructans represent a transient storage form. Fructans and sucrose are involved in cell expansion of grass leaves and likely serve as an osmoticum[76]. Sucrose may also substitute for K^+ as an osmoticum as was recently found for guard cells by Talbott and Zeiger[86]. The question of sucrose import into vacuoles merits further investigation. It needs to be clarified whether K^+ plays an essential role which cannot be substituted by other

mechanisms. The problem also has practical ramifications for K^+ nutrition of sucrose storing crops[49].

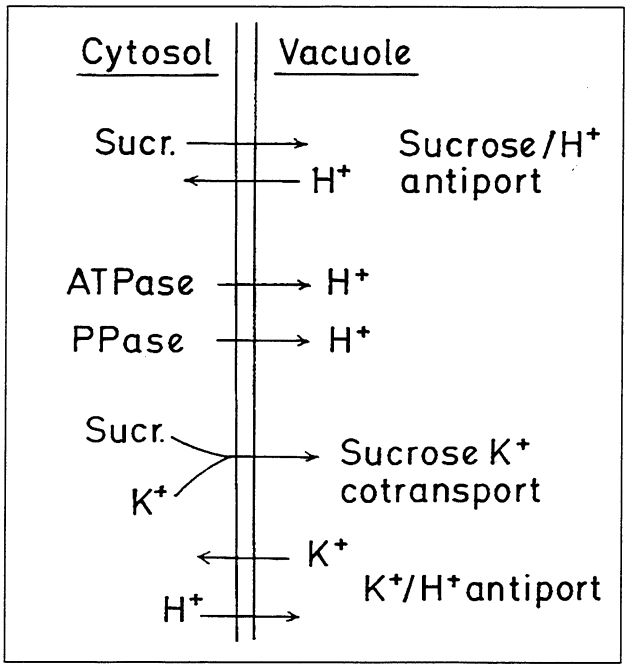

Figure 5. Scheme of vacuolar sucrose loading with ATPase and pyrophosphatase as proton pumps. Sucrose uptake is shown as occurring via sucrose/ proton antiport[10, 25] and as sucrose/K^+ cotransport[71].

Meristematic Physiological Sinks

The uptake of sucrose and amino acids from the apoplast into the cytosol of meristematic cells is driven by a plasmalemma ATPase which is stimulated by indole acetic acid[77]. Acidification of the apoplast from ATPase activity not only promotes the loosening of cell wall structures[21], but also provides the protons for the H^+/cotransport of sugars and amino acids across the plasmalemma. Heterotrophic cells are fed by the phloem sap which is composed (for most plant species) mainly of sucrose, amino acids, and K^+ (see **Table 3**). As mentioned above, K^+ uptake stimulates the proton pump and thus promotes the uptake of sugars and amino acids. The latter is required for protein synthesis, and the former serves as energy source and also provides organic carbon skeletons for biosynthesis.

The consumption of organic osmotica such as sugars and amino acids leaves K^+ as the most important inorganic osmoticum in meristematic cells, balancing the organic anions produced by the break-down of sugars and amino acids. Potassium plays a still more important role in proteins synthesis[40]. According to Wyn Jones and Pollard[91], there is good reason to assume that transcription as well as the the binding of tRNA to ribosomes requires K^+. Most important in this context is that these processes demand high K^+ concentrations in the range of 120 to 150 mM. Presumably, protein synthesis in meristematic cells is the most K^+ sensitive process. This suggestion is supported by experimental work carried out with whole plants. **Figure 6** shows the uptake and metabolism of ^{15}N labeled nitrate by young tobacco plants[37]. Plants sufficiently supplied with K^+ took up more labeled nitrate than plants with low K^+ supply. More impressive, however, is the finding that the percentage of labelled N used for protein synthesis was several times higher in the high K^+ treatment. It is evident from **Figure 6** that rather than nitrate uptake, or amino acid synthesis, it was protein synthesis which was most significantly influenced by K^+. It should be emphasized that the tobacco plants under the low K^+ treatment showed no K^+ deficiency symptoms. Analogous findings were observed for protein synthesis in roots and root nodules of *Vicia faba*[53] and *Medicago sativa*[18]. In the work with barley, saline conditions depressed the K^+ concentration in leaves with the consequence that protein synthesis in the shoots was much reduced. Increase in K^+ supply restored protein synthesis[32]. In this treatment the K^+ concentration in the shoots was 55 mmol/kg fresh matter. These results indicate that it is the K^+ concentration in the tissue, and presumably the cytosolic K^+ concentration, which plays a decisive role in protein synthesis.

All of the experiments noted above were undertaken with young plants; the ^{15}N applied was mainly incorporated in proteins within the growing vegetative tissue. This assumption is in agreement with results of Scherer et al.[75], who found

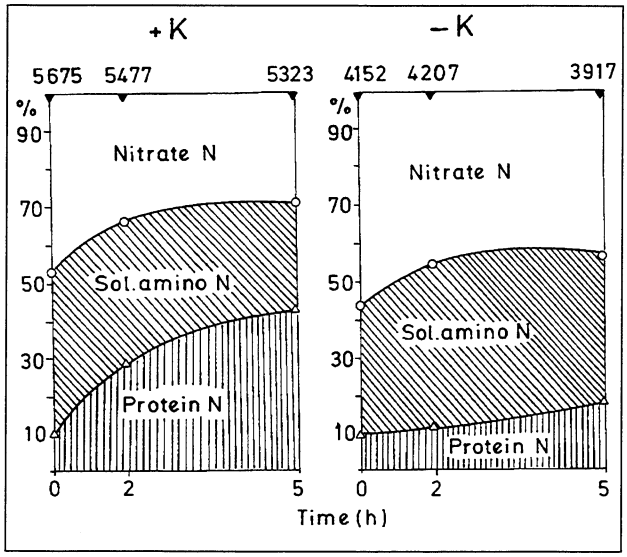

Figure 6. Total uptake of ^{15}N (nitrate) of tobacco plants from the nutrient solution with plants well and less well supplied with K^+, and the percentage of ^{15}N labelled nitrate, amino acids and protein. Plants of both treatments were at first grown at 1 mM K^+ in the nutrient solution. After 5 weeks of growth, K^+ was replaced by 1 mM Na^+ (-K), or maintained (+K) in the growth medium, and plants of both treatments were grown for an additional 12 days. ^{15}N labelled nitrate was then added to the growth medium of plants exposed to both treatments. Numbers at the top of each box refer to the total amount of ^{15}N (mg) per plant[37].

with whole young wheat plants that growth was the most sensitive process influenced by K^+ nutrition. At the beginning of growth retardation in the plants subjected to low K^+, the leaves contained higher glucose, fructose, sucrose, and fructan concentrations than the leaves of well supplied plants. This finding confirms that it was not supply of assimilate which affected growth process, but rather restricted protein synthesis resulting from too low a K^+ concentration in the meristematic cells.

Plant Water Status

Ionic K is an important osmoticum. Since it may occur in high concentrations in the vacuole and in the cytosol[35], its osmotic function is universal and contributes to a) the uptake of water from the soil solution by root cortical cells, b) adaption to saline conditions, c) retention of water in leaves, and d) maintenance of turgor pressure in specialized cells such as guard and motor cells[63], the phloem[52,84] and in most other plant tissues. In this respect, the turgor of growing tissues is of particular interest because these tissues are predominantly supplied by the phloem sap. Potassium is the most important inorganic osmoticum in phloem sap. Several researchers have reported that K^+ is essential for cell expansion particularly in combination with phytohormones[15,27,4]. Mengel and Arneke[47], working with whole plants of *Phaseolus vulgaris*, found that optimum K^+ nutrition significantly increases the turgor of cells of young leaves and is associated with cell expansion. The increase in turgor resulted from a decrease in the solute water potential due to a higher K^+ concentration (**Figure 7**). Cell expansion was negligible at a turgor pressure <0.5 MPa. This turgor threshold value is in agreement with data of Sionit et al.[83] for wheat. It thus appears that in growing tissues, K^+ not only plays a role in protein synthesis but also in cell expansion. Pfeiffenschneider and Beringer[65] reported that cell size in the medulla of mature storage roots of *Daucus carota* was much enlarged by an optimum K^+ supply. According to Beringer et al.[5], starch synthesis in potato tubers was not affected by a very high K^+ supply but the high K^+ concentration in the tuber cells increased the quantity of water retained per g tuber dry matter and thus decreased the starch concentration in tubers.

The osmotic function of K^+ is not a specific one; K^+ may be replaced by other osmotica, particularly Na^+ and sucrose. The substitution of Na^+ for K^+ depends much on plant species and only in natrophilic species does this substitution play a major role[45]. Even in sugar beet which is known as a natrophilic species, Na^+ may substitute for K^+ in leaf growth but not for the growth of storage roots[43]. This may be due to the fact that in the phloem sap, which supplies the sugar beet storage tissue, the K^+ concentration is high, and the Na^+ concentration is low. Substitution of sucrose for K^+ as an osmoticum can result in a waste of photosynthate. As reported by Tallbott and Zeiger[86], sucrose replaces K^+ in guard cells during a day's cycle. The osmotic functions of K^+ in guard cells and its impact on stomatal opening and closure are well known[35]. More recent research has shown that the plasmalemma of guard cells is well equipped with numerous K^+ channels[89].

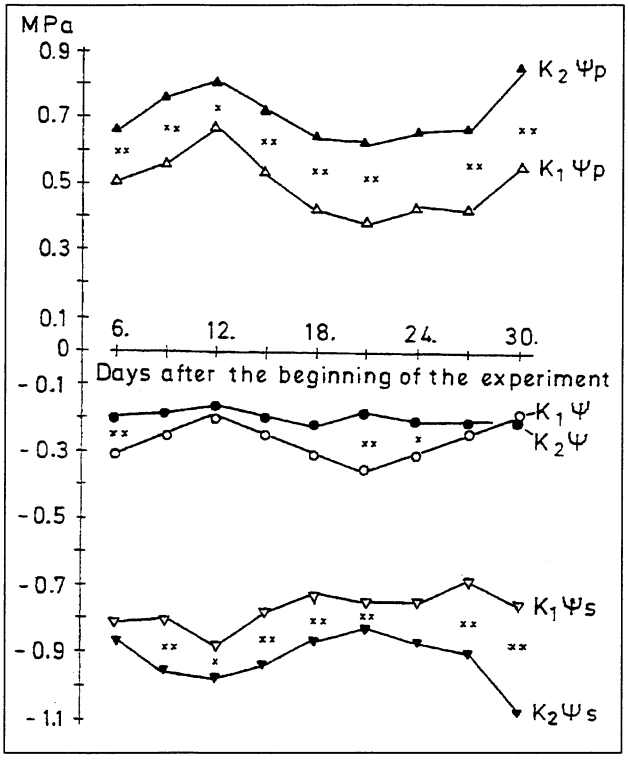

Figure 7. **Pressure potential (Ψp), water potential (Ψ), and solute potential (Ψs) in young leaves of *Phaseolus vulgaris*. K_1 = treatment with 0.1 mM K^+; K_2 = treatment with 4 mM K^+ in the nutrient solution[46].**

The various functions K^+ achieves for the plant water status are of practical importance because an optimum K^+ supply results in an efficient use of water by crops[7,49]. In this respect the finding of Pier and Berkowitz[67] merits particular interest. They reported that the depressive effect of water stress on CO_2 assimilation of wheat plants was much counteracted by a high K^+ supply.

Summary

Potassium ions are taken up by plant roots and distributed rapidly throughout whole plants because of very efficient uptake systems. These systems may be grouped into two major types: K^+ channels which allow a selective passive K^+ flux and K^+ transporters which mediate cotransport across membranes. Both uptake systems are driven by proton pumps, the activity of which is promoted by K^+. Potassium ion concentration in the xylem sap is 10 to 20 mM and 50 to 100 mM in the phloem sap. Long-distance transport of K^+ in the entire plant is therefore very efficient and K^+ can be quickly transported in acropetel and basepetal direction to all tissues.

The most important function of K^+ in the funda-

mental physiological source, the chloroplast of the actively photosynthesizing leaf, is the efficient transfer of light energy into chemical energy due to the increased pH difference across the thylakoid membrane. This general effect, together with the promoting influence of K^+ on phloem loading, provides an ample supply of sugars and amino acids to the physiological sinks and thus promotes the synthesis of proteins, carbohydrates and lipids as well as important molecules of secondary plant metabolism. The most sensitive effect of K^+ is its unique activation of protein synthesis in meristemtic tissues, which requires cytosolic K^+ concentrations in the range of 120 to 150 mM. In addition, K^+ is an essential osmoticum in elongating cells of growing tissues. Both effects, the impact of K^+ on protein synthesis in meristematic tissues and the impact on cell elongation, indicate the paramount role K^+ plays in vegetative growth.

The function of K^+ as osmoticum is of particular significance in various cell types such as guard cells and motor cells. The general osmotic function of K^+ in cortical root cells favors the exploitation of soil water and the adaption to saline soil conditions. In leaf cells, water retention is improved by K^+. Both processes, water uptake by roots and water retention by leaves, contribute to efficient water use.

Synopsis for Future Research Imperatives

Optimum supply of crops with K^+ is of utmost importance for crop production. This supply depends on K^+ fertilizer application and on the dynamics of K^+ in soils. On a global scale, it is often difficult to achieve optimal K^+ supply to crops, particularly on soils which are by nature of low fertility, such as the Oxisols frequently present in third world countries. These soils are poor in K^+ bearing minerals, their cation exchange capacity is low, and K^+ is easily leached out of the rooting profile. Under such conditions, K^+ fertilizer application should be made at frequent and low rates according to the crop development. Crop types should be developed which can take up K^+ rapidly during the early stage of growth and from which the crop may profit in later stages. Generally, determinate crops need K^+ primarily in the vegetative stage and less so in the reproductive stage. Investigations should be carried out on K^+ uptake systems, channels and transporters which enable the plant to take up high rates of K^+ over a short period and store vacuolar K^+ in large quantities.

On soils with high concentrations of K^+ bearing minerals such as micas, K^+ uptake systems are required which can depress the K^+ concentration of the soil solution to a very low level so that interlayer K^+ is released. It may be supposed that substantial differences exist between the various crop species in this capability. Mengel and Rahmatullah[58] reported that elephant grass (*Pennisetum purpureum*) could draw sufficient K^+ from the sand fraction of a mica rich Entisol on which other crops (maize, barley, wheat) suffered severely from K^+ deficiency. Such findings require elucidation.

World food production depends much on available water which in rain-fed agriculture frequently is the limiting production factor. On such locations it should be ascertained to what degree optimum K^+ nutrition may improve water efficiency and which processes are particularly affected by water stress. In this respect the finding of Pier and Berkowitz[67] is of particular interest; showing that under water stress high K^+ rates counteracted the negative effect of water stress on CO_2 assimilation. This observation needs further physiological elucidation and testing under practical conditions of farming.

In many parts of the world soil fertility is threatened by salinity. As shown by Cramer et al.[14], under such conditions more Ca^{2+} is required to counteract the deleterious effect of Na^+ on plant membranes. In addition, the surplus of Na^+ in saline media depresses the uptake of K^+ with the consequence of low K^+ concentrations in meristematic tissues and depressed protein synthesis[32, 33]. By means of modern techniques, such as genetic engineering, crop plant genotypes should be developed with K^+ channels equipped with a very high selectivity for K^+ and with a very low frequency of Na^+ conducting channels in the plasmalemma of cortical root cells. This example indicates that in future a close cooperation is needed between research in gene technology and crop physiology. The physiological, biochemical and biophysical obstacles which must be overcome in order to improve crop growth, particularly in problem areas suffering from salinity and low water availability, should be focused on in future work.

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Chapter 2:
Cytoplasmic Potassium Homeostasis: Review of the Evidence and Its Implications

R. Gareth Wyn Jones

Centre of Arid Zone Studies and School of Biological Sciences, U. W. Bangor, Bangor LL57 2UW, Wales, U.K.

Introduction

While the evolution of scientific hypotheses is rarely of major interest to active researchers, it may be timely after 20 years to review the concept of cytoplasmic K^+ homeostasis in plants and related ideas[24, 32, 33, 83, 86, 87] and their impact on our understanding of cellular and whole plant K^+ nutrition and of plant metabolism in general.

It is also instructive to cast our minds back not just 20 years, but to 1936. In that year Hoagland and Broyer[22] observed that the rate of K^+ uptake was regulated by the K^+ content of the roots—what would now be referred to as negative feed-back control. Since then this basic observation has been examined in detail by a number of authors[5, 13] . Similarly, Pitman and others[51] showed that K^+ uptake into shoots is regulated by the (K^+ and Na^+) content of the shoots. There is, therefore, a wealth of evidence for the tight regulation of tissue K^+ concentrations and that these, in turn, contribute to cellular osmo- and turgor-regulation in many instances[5, 31, 87]. Nevertheless, the mechanisms by which such regulation occur are unclear and indeed the recent data confirming, unequivocally, cytoplasmic K^+ homeostasis over a wide range of external and vacuolar K^+ levels[78] pose new challenges to our understanding.

In this chapter I wish (i) briefly, to review the hypothesis, (ii) to summarize the data obtained using the new triple barrel micro-electrode technique[78] because of its importance in validating the hypothesis, (iii) to emphasise the important insights gained by generalizing the hypothesis to symplasmic and phloem solute selectivity and transport, and (iv) to examine the implications of these generalizations to the relationships among metabolic activity, cytological structures, and solute accumulation in one illustrative tissue, namely the meristematic and adjacent elongating zone of the cereal root.

Review of Hypothesis

The hypothesis, as originally presented in 1976 and subsequently modified and elaborated, may be summarized as in **Table 1**.

The original hypothesis offered no coherent explanation for the K^+ specificity of the cytosol (cytoplasm). It rested on rather limited data from higher plants, depending heavily on the results of Dieter Jeschke's laboratory[26], but buttressed by a wealth of comparative animal data, summarized in Steinbach's model[68] and in Pitts[53] or Prosser[60]. The concepts were also motivated by the need to explain the apparent paradox of the high K^+ selectivity of halophytes even when the leaves are absorbing high levels of Na^+ and Cl^- to achieve gross leaf osmotic adjustment. Such halophytic plants also are more sensitive when grown in high K^+ salt media than in Na^+ salts in the 50 to 100 mol/m^3 range.

Table 1. Elements in K^+ homeostasis model.

1. Preferential cytoplasmic accumulation of K^+ (apparent concentration range *circa* 100-150 mol/m^3).
2. Preferential vacuolar accumulation of Na^+, Cl^-, organic acids, sugars, etc. up to several 100 mol/m^3 (cytoplasmic levels ranging from a few mol/m^3 to perhaps 30-50 mol Na^+ and Cl^- in some halophytes).
3. Preferential cytoplasmic accumulation of compatible organic solutes for osmoregulation or in dessicated tissue, e.g. embryos, pollen, etc. (occurs when ψ> 350 to 400 mosmol/kg or -0.8 to -1.0 M Pa).
4. Preferential cytoplasmic accumulation of K^+ (homeostasis) under K^+-depleted conditions. (Vacuolar solute potential generated by other salts or available organic solutes, e.g. sugars or amino acids).

Later, following Lubin and Ennis[35] and Weber et al.[79], in animals, cytoplasmic K^+ -selectivity was attributed quantitatively to the ionic requirements of the translation step of protein synthesis[11, 83]. In the mid 1980s the hypothesis was extended to account for K^+ behaviour under K^+-limited conditions and for the well-known curves relating growth to K^+ tissue content and K^+ supply, including the phenomenon of "luxury consumption", **Figure 1** (compare Marschner[40] and earlier textbooks). The proposal that cytosolic K^+ levels are preferentially maintained in an apparent concentration range of 100-150 mol/m^3 at the expense of a falling vacuolar K^+ level, leads to the following simple interpretation. The steep dependence of growth on K^+ tissue levels and supply (see **Figure 1**) was associated with an inability to meet the rigorous biochemical (cytoplasmic) requirement for the ion, while the shallow dependence (luxury consumption) was considered to reflect the more reflexible, biophysical role of K^+ salts in generating the vacuolar solute potential (Ψvac)[32, 33]. A number of mobile solutes are capable of fulfilling this function.

This hypothesis suggests an explanation for both the high K^+ selectivity of Na-salt accumulating halophytes and their greater sensitivity to modest external K^+-salinities. While such plants are capable of fulfilling their cytoplasmic K^+-requirement, they probably lack the ability to sequester high K^+ concentrations in their vacuoles without a concomitant increase in the cytoplasmic K^+ to toxic levels.

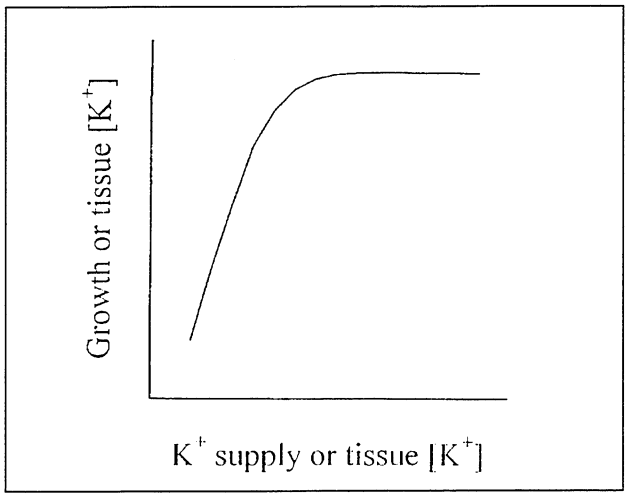

Figure 1. Schematic relationship of: (i) growth (y axis) versus tissue [K^+] and (ii) tissue K^+ (y axis) versus K^+ supply.

Table 2. Cytosolic (cytoplasmic) solute concentration ranges[1].

pH	7.2 - 7.4
K^+	100 - 140 mol/m^3
Na^+	<5 - 30 mol/m^3
Mg^{2+}	2 - 4 mol/m^3
Ca^{2+}	0.1 - 2 mmol/m^3
HPO_4^{2-}	5 - 20 mol/m^3
NO_3^-	4 - 5 mol/m^3
SO_4^{2-}	<1 mol/m^3
Cl^-	<10 - 50 mol/m^3
Organate^{n-}	2 - 10 mol/m^3
Protein^{n-}	see[2,3]
ψCyt[4,5]	300 - 350 mosmol/kg

[1]Apparent concentration range not activity.
[2]Cation charge = Anion charge.
[3]As the permeability of protein^{n-} is very low, the cytosol is a Donnan phase in relation to the vacuole.
[4]Cytoplasmic compatible solutes accumulated at solute concentrations greater than circa 400 mosmol/kg; these include proline, glycinebetaine, sorbitol, mannitol, glycerol, pinitol and various cyclitols and dimethylsulphonio propionate.
[5]Refs.: 32, 33, 83

While this hypothesis was principally concerned with K^+ nutrition, it was apparent that the concept had much wider implications for the behaviour of a great range of inorganic and organic solutes. The hypothesis can be summarized in the phrase: "the promiscuous vacuole and the fastidious cytoplasm." The 'fastidiousness' of the solute specificity of the cytoplasm, as well as the highly conserved, biochemical behaviour, is observed uniformly throughout all eukaryotic cells and is indeed found in Eubacteria (only the Archaebacteria behave dramatically differently). It is possible to claim that 'cytosol is a cytosol is a cytosol is a cytosol', to misappropriate Gertrude Stein's famous aphorism. The best estimates for the ranges of cytosolute concentrations (ignoring any compatible solute accumulation) are given in **Table 2**.

The contrast between the "conserved" solute behaviour of the cytosol and also, where data are available, cytoplasmic organelles, e.g. chloroplasts (for example, 64) and the enormous flexibility of vacuolar solute composition and concentrations is striking. It is reflected in such historic data sets as those of Mott and Steward[44] studying carrot explants, some of whose results are reproduced in **Table 3**. The differences in solute concentrations in the different explants can be interpreted as reflecting (a) the degree of vacuolation of the tissue and (b) the flexible and opportunistic character of vacuolar accumulation.

Yet volumetric and cytological relations between cytoplasm and vacuole mean not only that the vacuolar contents dominate the whole tissue analyses in all but meristematic zones and specialised structures, e.g. pollen and stamens, but raise specific difficult issues. How, for example, are the solute contents of the two compartments of such differing volumes regulated, partly independently? (differing ion specificities) and partly in coordination? (preventing the Donnan swelling of the cytosol) while maintaining osmotic and hydrostatic equilibrium across the tonoplast? The main ion channels and pumps across both the plasma membrane and the tonoplast are being characterized. Their ionic specificities and orientation are consistent with the model, but as yet do not explain how the various regulatory functions are achieved (see 78).

It is important to recognize and acknowledge that the hypotheses elaborated above depend heavily on the ideas and results of others, especially Steinbach and Schoffenields (from marine animal physiology), Brown and Borowitzka (fungal physiology), and plant physiologists, Dieter Jeschke, Horst Marschner, Michael Pitman, George Stewart, Barry Osmond, Hank Greenway and Tim Flowers, as well as my colleagues in Bangor and Rothamsted, whose work is quoted extensively in this chapter. In the last 20 years a number of apparent anomalies have been eradicated as definitive evidence supporting the model has been obtained, e.g. in *Dunaliella salina*[1]. The extensive studies of the Purdue group[46] have also added significantly to the weight of supporting evidence. In **Table 3**, a summary of analytical data related to K^+ compartmentation, mainly in higher plants, is presented.

Some of the papers presenting results at variance with the hypothesis (e.g. 20, 66) are either technically suspect, e.g. use the freeze substitution methodology, or are internally inconsistent if osmotic and turgor relations as well as ionic data are considered. Nevertheless, it is possible that lower K^+ levels may be accommodated in the cytoplasms of mature halophyte leaves of limited metabolic activity[10]. Despite the consistent pattern, no technique has produced precise, unequivocal values for the cytosolic K^+ activity (a_{K^+}) nor the capability of relating *in vivo* K^+ activity or concentration to metabolic events. Furthermore, the semi-quantitive

Table 3. Some estimates of cytoplasmic and vacuolar (serum) concentrations of K^+.

Species and tissue	K^+ concentration Extn	Cyt	Vac	Ref
	- - - - - - - - - (mol/m³) - - - - - - - - -			
Higher plants				
Hordeum vulgaris, root cortical cells	2.5	92	79	53
H. dist, root apical cells	0	110	20	26
H. vulgare, root cortical cells	0	71-119	9-12	52
H. vulgare, root cells	0.01	133	21	42
	0.1	140	61	
H. vulgare, root meristematic cells	0	194	—	23
	6.0	200	—	
A. thaliana, root cells	0.01	115	40	36
Pisum sativum, root cells	10	43	122	8
P. sativum, epicotyl cells	1.0	145-193	54-56	37
	10.0	160-180	78-79	
Avena sativa, coleoptile	10.0	140-215	155-190	50
Allium cepa, root cells	1.0	100	83	38
Z. mays, root	1.0	99-108	—	6
Acer pseudoplatanus, cell suspensions	7.0	175b	70	63
Z. mays, root	6.0	129-162	54-62	18
Suaeda maritima, leaf cells	—	0-16[c, d]	11-24	19
Atriplex spongiosa, apical cells		200		70
bundle sheath cells		150	30	
Carrot callus, growing incipient vacuole	u6	(110)	—	44
growing vacuolated cells	u6	?	(68)	
Lower plants				
Chara australis	0.1	160[b]	64	76
Chara australis		112	112	71
Nitella pulchella		101	116	
Conocephalum conicum	0.1	101b	—	75
	10.0	108b	—	
	extn.	cyt.		
Animals	(serum)	(muscle)		
Homo sapiens	4	160		54
Frog (Rana)	3	126		59
Mytilus edulis	13	158		

[a]concentration (c) x activity coefficient (f) = activity (a)
[b]concentration entrapolated from activities determined by ion electrodes
[c]values for cytoplasm determined with beam partially located in cell wall or vacuole
[d]see ref. and text for comments on freeze substitution methodology

model, although correlating well with the selectivity and polarity of the plasma membrane and tonoplast fluxes, offers few regulatory insights.

The recent work of David Walker, with Tony Miller and Roger Leigh, in first developing and then cleverly exploiting a triple barrelled micro electrode capable of detecting simultaneously, a_{K+}, a_{H+}, pH, and E_M is therefore of major significance. It allows unambiguous cytosolic K^+ activities to be determined as well as the vacuolar values. Both the technique and the results are dealt with in detail in the chapter by Leigh et al. in this volume.

In **Figure 2**, the relationships among cytosolic, vacuolar and tissue K^+ activities in wheat roots are nevertheless reproduced because they provide such compelling evidence for the hypothesis under discussion. The cytosolic a_{K+} does not vary over a six-fold change in the vacuolar concentration. The homoeostatically maintained cytosolic K^+ activity may be used to calculate the apparent chemical concentration if the activity coeffecient is known or a realistic value assumed. While the accurate cytosolic value is not known, it is probably relatively low, perhaps 0.6 to 0.7, given the low values recorded for unicationic multianionic salts[62] and the importance of HPO_4^{2-} and $protein^{n-}$ (**Table 2**) as counter anions in the cytosol. This suggests cytosolic chemical concentrations in the range 100-140 mol/m³, in excellent agreement with the hypothesis and the *in vitro* biochemical evidence.

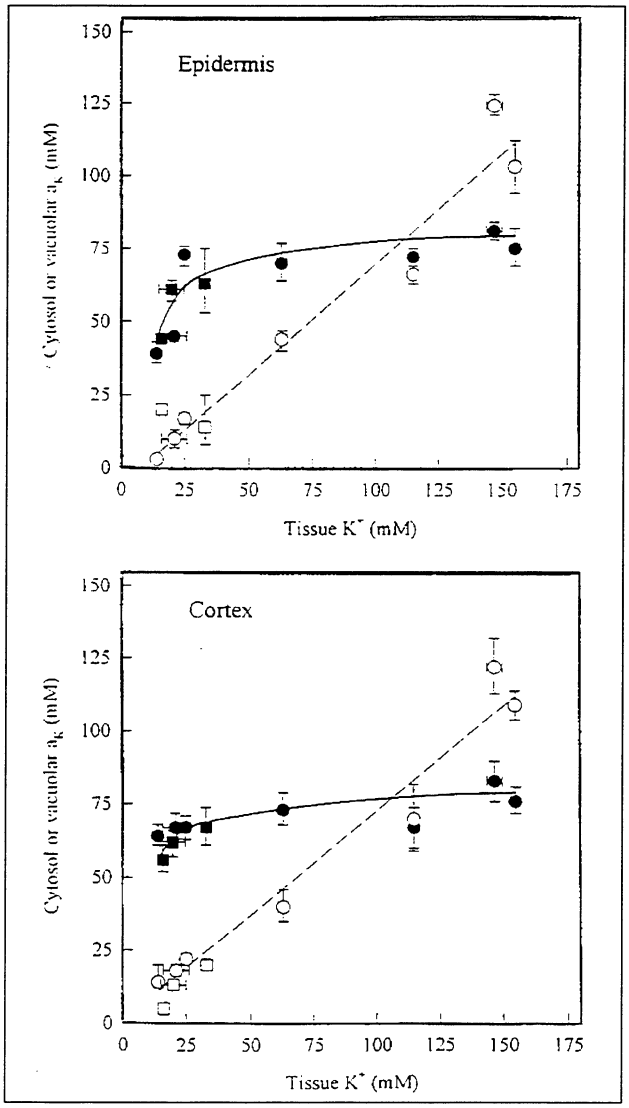

Figure 2. The relationship between tissue K^+ concentration and vacuolar (open symbols) and cytosolic (solid symbols) a_k in epidermal and cortical cells of low (square) and high salt (circles) barley roots. (See ref. 78 and chapter 7 in this volume).

The combination of the triple barrelled micro electrode technique with the vacuolar micro-sampling and micro-analytic techniques developed by Deri Tomos and his colleagues (see reference 73) offer exciting opportunities to explore how the osmotic and Donnan and electrical equilibria are regulated across the tonoplast, remembering that the cytosol with a fixed anionic charge on the cytosolic proteins is a Donnan phase. More reliable values for the activity and osmotic coefficients of major solutes in plant cells could also be obtained by combining these new techniques.

A number of studies have established that protein synthesis is curtailed in K^+-deficient tissues and that such tissues accumulate free amino acids[29, 43, 49]. These observations are compatible with the *in vitro* biochemical evidence showing a requirement in eukaryotic systems for 120 to 150 mol/m^3 K^+ for efficient polysomal translation[11]. However, there is no quantitative evidence in plants showing that an *in vivo* decline in protein synthesis is specifically and quantitatively related to the cytosol a_{K^+}, as predicted by the model. The triple barrelled micro electrode will allow this prediction to be tested with quantitative results to establish whether the steep phase of the K^+ response curve is indeed related to a specific decline in K^+ biochemical activity.

Symplasm and Phloem

The significance to higher plant metabolism of the extension of cytosol into a symplasmic continuum via plasmadesmatal connections is well documented (e.g. reference 30) as are its implications for K^+ and other nutrient transport, for example, in relation to trans-root fluxes or secretory cells (e.g. reference 3). The strong evidence now available for high K^+-selectivity and homeostasis in the cytosol can clearly be extrapolated to the symplasm in general and potentially to any cell type or tissue dependent on symplasmic transport for its solutes. An important observation in this context is that of Stelter and Jeschke[69] who found in the halophyte, *Altriplex hortensis*, that xylem-loading has a high $Na^+ > K^+$ selectivity in contrast to the $K^+ > Na^+$ selectivity found in glycophytes. In the absence of this evidence, it would be difficult, if not impossible, to reconcile the high leaf Na^+ and Cl^- levels of dicotyledenous halophytes, the ionic demands of the symplasmic phase of trans-root transport, and low cytosolic Na^+ and Cl^- activities in the root cortical and stelar cells as predicted by the hypothesis and confirmed by experimental data (e.g. 71).

Raven[61] originally emphasized the close relation between symplasmic and phloem ion composition and concentration. While his paper concentrated primarily on H^+ and Ca^{2+}, the same insight can be extended to other ions and to organic solutes[80]. In **Table 4**, data on the ionic composition of phloem are presented to support this contention but additional evidence would be desirable using more modern techniques.

Given the evidence of the fundamental ionic continuity of cytoplasm, symplasm and phloem, it is possible to extrapolate the "fastidious cytoplasm" hypothesis to explain, interpret and illuminate many aspects of whole plant nutrition.

Table 4. Phloem electrolyte composition.

	Mol/m^3
K^+	20-112
Na^+	0.06-30
Cl^-	5-30
HPO_4^{2-}	3-10
Ca^{2+}	0.2-0.5
Mg^{2+}	2-4
Organic Acid $^{n-}$	~40

Major organic phloem transport solutes include sucrose, pinitol, mannitol, and sorbitol.

The chemical composition of phloem sap and various tissues from *Aster tripolium* (**Table 5**) shows preferential K^+ accumulation and Na^+ and Cl^- exclusion in the phloem sap over a huge range of external electrolyte concentrations. Osmotic adjustment is achieved partly by sucrose accumulation although a large osmotic contribution is unaccounted. It would be of great interest to know if this deficit in the phloem sap of sea water grown plants is compensated for by solute (glycinebetaine), accumulation. As might be anticipated, the largely phloem-fed florets of the salt marsh plants are relatively high in K^+ and low in Na^+ and Cl^- while accumulating betaine and sugars, whereas leaf (largely vacuolar, xylem-fed) samples are dominated by Na^+ and Cl^-. A similar pattern is revealed by the low salt (NaCl) levels found in salt-grown barley seeds[16], the high glycinebetaine levels of wheat aleurone and embryonic tissues[2], the low salt content of salt-grown tomatoes derived from wide hybridization[64] and the high glycinebetaine, proline and pinitol levels found in pollen and other reproductive tissues of different species[14, 84]. Dramatic data were reported recently by Girousse et

Table 5. Chemical composition of *Aster tripolium* tissues[a].

	Phloem Sap			
	Fresh-water	Sea-water	Leaves[b]	Florets[b]
Measured osmolality (mosmol/kg)	599	1544		
K^+ (mol/m^3)	86	100	72	133
Na^+ (mol/m^3)	0.35	31	360	56
Cl^- (mol/m^3)	5	28	320	51
Sucrose equiv. (mol/m^3)	302	672	—	—
Free sugars (mol/m^3)	—	9	67	
Glycinebetaine (mol/m^3)	—	—	18	82
Amino acids (mol/m^3)	—	—	12.8	58
Osmality accounted for (mosmol/kg)	460	920	—	—

[a]See refs. 8, 14, and 82
[b]Samples collected from a salt marsh

al.[12] showing selective accumulation of the compatible solute proline in alfalfa phloem sap when the osmotic potential exceeded -0.8 MPa, again in line with the hypothesis. Similarly, there are extensive data showing that the solutes accumulated in both shoot and root apices follow the same 'symplasmic' pattern.

The extensive and detailed research of Jeschke, Pate, and their colleague on solute circulation in higher plants also sustains the same interpretation. Indeed their work does much more, as it shows that the degree of selectivity in phloem for K^+ over Na^+ varies from one plant species to another [25, 80]. In **Figure 3** data on ion circulation in barley are reproduced. They show the importance of K^+-selective phloem transport to the preferential accumulation of ion in both the shoot and root apices, reinforcing a wealth of static analyses previously reported (e.g. 15, 26, 45, 70, 71). Of particular interest, therefore, is the evidence for the importance of phloem transport of K^+ in maintaining the observed K^+ levels in root tips. This occurs even though external K^+ is being taken up by the root epidermal and, possibly, cortical cells for subsequent xylem transport to the expanding and the expanded, highly vacuolated, photosynthetically-competent leaves and, in the final analysis, retransport to all other tissues. These results also show that Na^+ is retained in the older leaves so that the total leaf K^+/Na^+ falls from 42.5 in leaf 4 (young) to 3.3 in leaf 4 (old). The high K^+/Na^+ ratio of leaf 4 and of the root tissue is more a reflection of phloem discrimination than the K^+/Na^+ selectivity in the uptake or xylem transport phases. In a species such as Lupin, the capability for phloem K^+/Na^+ discrimination is lower, which may be one element in the greater salt sensitivity of that species[25]. Similarly, Lessani and Marschner[34] observed that the percentage of Na^+ retranslocated and excreted from roots was much smaller in salt-tolerant sugar beet than the salt-sensitive bean, presumably due either to better vacuolar retention and/or greater phloem exclusion of Na^+ or both. A very similar phenomenon may well be involved in the well documented variability in ability of Na^+ to "spare" the K^+ requirement of crops in fertilizer trials (at the high 'biophysical' concentration range, **Figure 1**) and the Na^+ stimulation of mainly fresh weight growth in some species[39].

Potassium Supply Route, Growth and Homeostasis

Early in this chapter, although the important role of K^+ as a contributor to cellular osmotic potential and turgor generation was recognized, it was not discussed in any detail. In the final section of this chapter I wish to explore a number of interrelated issues in greater detail, especially the relationships between tissue K^+ concentrations, turgor generation and homeostasis and cytoplasmic K^+ selectivity observed in some tissues but not apparently in others.

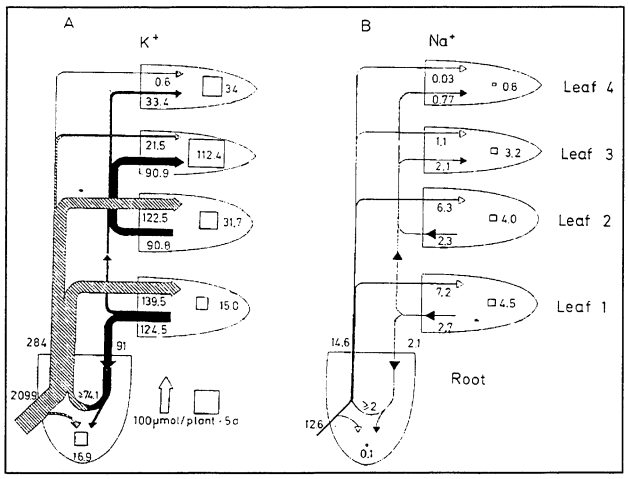

Figure 3. Rate of K^+ (A) and Na^+ (B) flow between single leaves and the root of barley (cv. California Mariout) at 1 mol/m³ K^+ and 1 mol/m³ Na^+. The figures represent ion movements and increments in individual organs. Thickness of arrows is drawn in proportion to transport rates: xylem hatched, phloem black. Squares in leaves and roots represent ion increments due to growth and/or to increases in tissue ion concentrations. (See ref. 80 for details.)

The strong K^+ selectivity of meristematic, young tissues was demonstrated clearly in **Figure 3**. The same phenomenon is revealed, rather differently, in a simple set of experiments[56] (Owen and Wyn Jones, unpublished observations) in which the longitudinal osmotic potential and K^+ profiles were determined in 6-day-old wheat seedlings grown either in 'low-salt' $CaSO_4$ only or 'high-salt' i.e. in KCl plus $CaSO_4$ media (see 7) (**Figure 4A and 4B**). Even such whole tissue, therefore "highly vacuolated" data, reveal some interesting trends. There is a relatively strong conservation of both osmotic potential (π) and K^+ in the root apical centimeter and the basal portion of the shoot. In low salt seedlings both sap π and K^+ fall rapidly away from these partially meristematic/elongation zones, especially in roots.

There is also a very large step in sap and K^+ concentration at the root/shoot nodal interface which raises a number of interesting issues. From the work of Pritchard and Tomos using similar material, it is also clear that in wheat and barley seedlings grown in the standard unsalinized media, the turgor pressures of leaf cells are significantly higher, at 0.8 to 1.0 MPa[57], than those observed in the root cortical cells of the same plants (0.3 to 0.65 MPa). Given the gradients shown in **Figure 4**, the size of the seedlings, and the culture regime, it seems probable that these differences in osmotic and turgor pressures and K^+ concentrations of mature leaf and root cells reflect intrinsic properties of the cell rather than a transpiration-induced difference.

The pressure probe and micro-analytical techniques allow the apparent conservation of osmotic potential (280-300 mosmol/kg) and K^+ (32-65 mol/

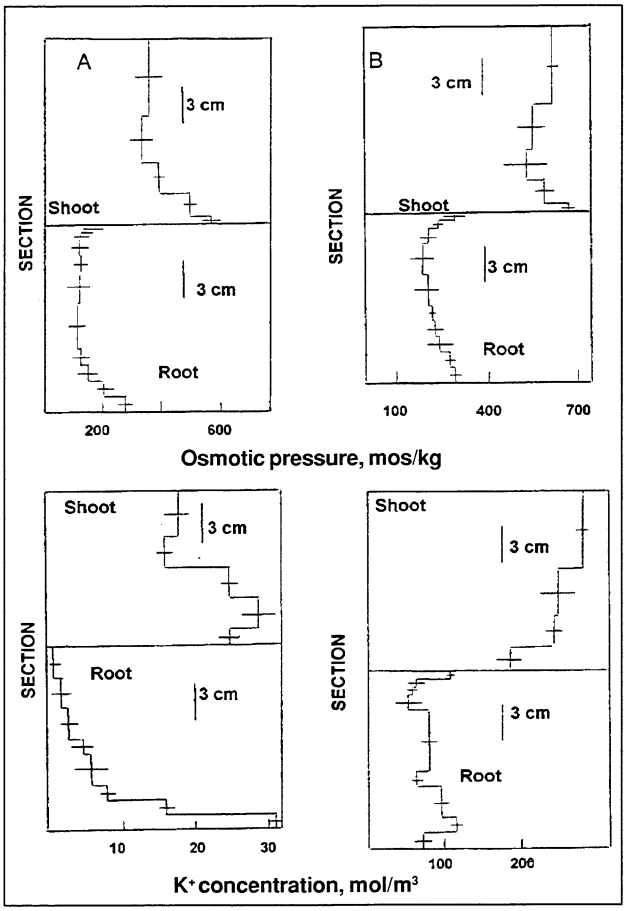

Figure 4A. Longitudinal π and K^+ profiles of 6-day-old low salt (0.5 mol/m³ $CaSO_4$) wheat seedlings. Length of section indicated on y-axis.

Figure 4B. Longitudinal π and K^+ profiles of 6-day-old high salt (10 mol/m³ KCl + 0.5 mol/m³ $CaSO_4$) wheat seedlings. Length of section indicated on y-axis.

m³) in the terminal segments (circa 1 cm) of 'low' and 'high' salt roots to be explored in greater detail. Such studies have been carried out primarily to explore the control of root cell elongation. However, a reexamination of these data suggests further implications for our understanding of K^+ nutrition.

It was observed[59] that the rate of root elongation in low salt wheat seedlings could be manipulated readily by the addition of low (a few mol/m³) concentrations of simple salts to the growth medium. As shown in **Table 6**, the fastest growth of the young seedling roots occurred in a 'low salt' ($CaSO_4$)-only medium. However, the growth rate is independent of turgor pressure even when measured in the zone of the maximum rate of elongation. This zone could be identified both by cytological studies and by measuring the local elongation rate every millimeter from the root tip. A very tight homeostatic regulation of turgor pressure independently of the elongation rate is a consistent feature of a number of studies[4, 57, 58, 66]. While this portion of the root also tends to accumulate K^+ selectively to a rather constant concentration (see **Figures 3 and 4** and ref. 26), both the turgor pressures and K^+ contents of cells 3 to 4 cm from the meristem are highly variable. In those cells and those in the main cortical section of the root, the turgor and osmotic pressures change in response to the availability and uptake of mobile electrolytes or organic solutes, for example, being very high in KNO_3-grown cells but very low in $CaSO_4$ or in $CaSO_4$ plus Na^+ salt-grown cells. As has been known for several decades (e.g. 53), these latter tissues, deprived of readily available external solutues, accumulate simple sugar and/or organic acids as major osmotica[44].

Table 6. Differential relationships between elongation growth and turgor pressure in wheat roots[a].

Growth solutions[b]	Growth rate	Turgor pressure: % of control	Turgor pressure: 3 mm from meristem	Turgor pressure: 32-40 mm from meristem
	(mm/24 h)		(MPa)	
$CaSO_4$	32 ± 0.6 (40)[c]	100	0.63 ± 0.02 (10)	0.30 ± 0.01 (10)
$CaSO_4$ + NaCl	30 ± 0.5 (40)	94	0.67 ± 0.01 (7)	0.35 ± 0.01 (11)
$CaSO_4$ + Na_2SO_4	22 ± 0.6 (40)	69	0.63 ± 0.01 (10)	0.30 ± 0.01 (10)

[a]See ref. 59
[b]0.1-1.0 mol/m³ concentration
[c]Figures in brackets refer to number of recordings

The general picture that emerges, therefore, is of tight turgor regulation in the elongation zone where turgor pressure is an essential prerequisite to growth, although the rate of elongation is itself determined by changes in the rheological properties of the walls, not by the modulation of the hydrostatic pressure[4, 57]. As there is a convincing weight of data supporting the general hypothesis of cytosolic but not vacuolar K^+ homeostasis, it might be tempting to speculate that K^+ uptake from the apoplast might be a part of the mechanism of turgor regulation in very young cells, as indeed appears to be the case in bacteria[21]. Although there are no direct data on the cytosolic K^+ levels in the elongating zone, recent work on single cell turgor, osmotic pressure, and solute concentrations of individual vacuoles of epidermal and cortical cells reveals an interesting but complex picture. This work was carried out for technical reasons on maize roots[58].

The tight regulation of turgor (0.48 ± 0.082 MPa) was again observed even in roots exposed for 24 hours to an osmotic stress of 400 mol/m³ (-0.96 MPa) mannitol, the cells in the elongation zone being able to achieve total osmotic and turgor compensation (**Figure 5**). The vacuolar K^+ concentrations were highest in the youngest (smallest) cells and fell quite rapidly as the cells (vacuoles) expanded, even when adequate K^+ was available in the external medium. A similar behavior was found in control and osmotically-challenged roots. Increased levels

of hexose, amino acids, and mannitol absorbed from the external medium made large contributions to osmo-regulation and turgor homeostasis in the plants, although about 20 percent of the osmotic pressure could not be accounted for by the measured solutes. The preferential accumulation and the apparent concentration of K^+, largely independently of the external osmolarity and K^+ supply, in the youngest, least vacuolate cells, fits well with previous observations and the general hypothesis. It is reasonable to suggest that the conservation of the K^+ content of these cells and of the meristematic cells is related to their intense biochemical activity (cell division, protein synthesis, respiration, cell wall synthesis etc.). It may well be that the solute content of young vacuoles mirrors that of the cytosol from which they emerge. However, there is clearly no correlation between vacuolar K^+ and turgor through the elongation zone and nothing to link K^+ directly to turgor regulation in what is clearly a critical phase in plant growth and development. Efforts to detect clear evidence for either turgor-regulated K^+ ($^{86}Rb^+$) influxes or turgor-modulated changes in cell membrane potentials in the terminal few mm of wheat roots have been unsuccessful (Frick and Wyn Jones, Miller and Leigh, unpublished). Thus there is nothing to indicate that the mechanism of turgor homeostasis is associated with a turgor-regulated H^+-efflux or K^+-influx at the plasma membrane. It seems unlikely, therefore, that either the type of turgor-regulated K^+ fluxes found in bacteria or the turgor-regulated electrogenic H^+-efflux observed in red beet storage tissue[88] can account for the tight turgor homeostasis in the root elongating zone.

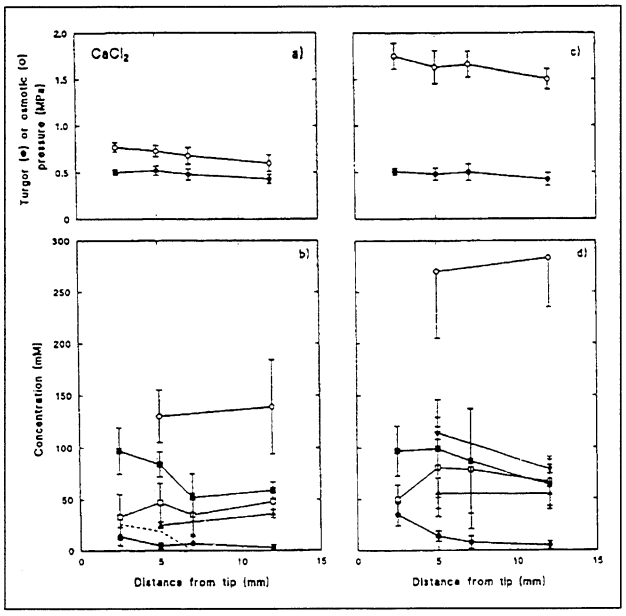

Figure 5. **Water and solute relations of maize roots. Turgor, osmotic pressure and individual solute concentrations of individual epidermal and cortical cells. Seedlings grown in 0.5 mol/m^3 $CaCl_2$ continuously (a) and (b), or for the 24 hours prior to analysis with 400 mol/m^3 mannitol (0.96 MPa) (c) and (d). The symbols in (a) and (b) denote the following: turgor (●), and osmotic pressure (O). The symbols in (c) and (d) denote the following solutes: K^+ (■), Cl^-(•), phosphorus (●), hexose (O), total amino acids (▲). and mannitol (▼). (See ref. 58 for details.)**

The results described above, together with other recent observations, suggest that a radically different explanation should be considered. In this the activity of the cells in the meristematic and adjacent elongating zones is viewed as being largely buffered from the external medium because of the orientation of the symplasmic transport pathway and its dominant role in nutrient supply. In such a model, direct solute uptake from the apoplast or the external medium would be relatively unimportant despite the rapid hydraulic contact between root tip cells and the external medium[57,85]. Jeschke and Wolf[27] were able to demonstrate in *Ricinus* that external K^+ supply is not required to maintain the root apical K^+ concentration at the high level which is needed for growth.

Not only do the results already outlined suggest that external electrolytes have only a minor role in turgor regulation and osmo-regulation in elongating maize roots, but other studies in maize show that the time course of turgor recovery after an osmotic challenge is more rapid in the proximal than in the distal part of the elongation zone. Since the solutes that are accumulated are characteristically cytoplasmic-symplasmic-phloem solutes, these observations imply that the distal zone is preferentially supplied. These results indicate that the phloem and interrelated symplasmic elements play a vital role in solute supply even beyond the protophloem. A technique for the real-time imaging of phloem unloading into Arabidopsis root tips using the fluorescence probe[56] carboxy fluorescein has been developed[47]. The rapid translocation of the dye to the root tip was observed, followed by unloading into discrete concentric files of cells. The two prominent unloading zones corresponded with two protophloem files of seive elements. Of particular interest to this discussion is the observation that symplasmic transport followed the unloading into the elongation zone with basipetal transport into the more mature cells. In light of studies of root tip sugar transport, and also Pritchard's[56] results, Farrar et al.[9] also concluded that preferential symplasmic transport to the meristem followed by basipetal loading of the more mature cells best accounted for their observations.

The basic concept, which can perhaps be termed the "fountain model", suggests that root meristems are supplied preferentially with sugars, amino acids, inorganic nutrients etc. as the raw material for cell division, metabolism, and turgor generation by directionally orientated symplasmic transport from the base of the sieve tubes. Once the requirements of these cells are satisfied, the root then "back fills" sequentially into first the proximal then the distal portion of the elongation zone. As elongation and associated vacuolation occur, the sol-

ute specificity of the vacuolar solutes may gradually change to reflect the increased flexibility or "promiscuity" of this organelle and the increasing availability of exogenous or xylem-derived solutes. For example, as first observed by Jeschke and Stelter[26] and shown in **Figure 5**, the initial phase of vacuolation uses K^+ salts, while Na^+ is accumulated a little later, even in the vacuole of the relatively salt tolerant species, barley.

This model further implies that the tight link between turgor and solute accumulation, which is an essential requirement of homeostasis in an expanding system, is not due to a turgor-regulated influx from the apoplast as this author, for one, certainly envisaged. Rather it is the symplasmic flow that must be turgor-controlled, possibly by some plasmadesmatal-gating mechanism. The ability of plasmadesmata to close (seal) rapidly when turgor is lost in an adjacent cell is well known[28, 48], and more recent authors have suggested this is due to pressure-mediated plasmadesmatal-gating.

Although there are few data, it may be logical to speculate that higher plants (sugar beet) have turgor-regulated plasma membrane fluxes (e.g. H^+ flux mechanism) in tissues depending on apoplastic supply and requiring tight turgor regulation. In contrast, turgor-gated plasmadesmatal fluxes would occur in symplasmically fed tissues such as root apices. Either system could contribute to cytosolic K^+ homeostasis although in different ways.

Not all plant cells, however, control their turgor tightly[31]. In some, such as more mature root cortical and epidermal cells, turgor homeostasis is weak, although cytosol $^{K+}$ is still tightly controlled. As discussed previously[28], this flexibility in the behavior of the main body of the root may be a mechanism to ensure that carbon, nitrogen, and other nutrients are directed to root growth and the exploration of fresh soil when water or nutrients are in short supply or potentially toxic. Cortical osmoregulation would be an expensive diversion of photosynthate as the upper horizons of a soil dry out or are exhausted and both mass flow of water and diffusive supply very restricted.

It has become clear that data on mineral nutrition[27, 41], phloem transport[6], cytology, turgor and osmotic regulation[58], and carbohydrate supply are all combining to provide an explanation for root tip metabolism which emphasizes its dependence on symplasmic supply with a characteristic solute signature which is relative independent (other than in hydraulic terms) of its immediate exterior.

If this working hypothesis is accepted, then one question that arises is how, in the elongating zone of the root or shoot, the cell is capable of integrating solute supply from two possible sources in a biphasing system of different solute specificities while maintaining turgor constant? The concept further emphasizes the importance of the tissue structures within which the transport functions are operating[75].

It is also interesting that the turgor pressures observed in expanding and mature shoot and root cells of the same well watered plants are so different, leading to the possibility of genetically determined different 'set points' in the two elongation zones of the same plant. Indirect selection for turgor pressure has been observed in beet[74] and barley[17]. Thus, there is a possibility that the median turgors of cell types in a given plant, as well as in different species, is under specific genetic control.

Finally, returning to Hoagland and Broyer[22], it is still not clear how the negative feedback from the total tissue K^+, i.e. the vacuolar K^+, to regulate the plasmalemma influx into the cytosol can occur. The models of Glass[13] and others are improbable and a trans-cytosolic regulatory message seem more likely. Similarly, even though such cells only regulate their turgor loosely, it is again not clear yet how they are able to integrate the vacuolar solute composition, be they electrolytes or non-electrolytes, and combine solute flexibility and opportunism with volume and osmostic control across the tonoplast.

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Chapter 3:
Molecular Characterization of Plant Potassium Channels

Gerald A. Berkowitz, Jiong Ma, Huixian Tang, Xiao Zhang, Zhiwei Fang, and Aurea C. Vasconcelos

Department of Plant Science, U-67, 1376 Storrs Rd., University of Connecticut, Storrs, CT 06268-4067

Introduction

The molecular characterization of ion channels and analysis of the structure:function aspects of this class of proteins is one of the more active areas of biological research today. An appreciation of the advances in our understanding of the mechanistic aspects of transport facilitated by these proteins is predicated on an understanding of some basic concepts of transport of charged particles across biological membranes. Therefore, this chapter will start with a presentation of some "core" concepts relevant to transport. After presentation of this background information, our current understanding of the molecular structure of plant K channels will be reviewed. This review will focus on the few plant K channels which have recently been cloned. The cloning and sequencing of these channels native to plants have led to an initial understanding of the structure of this family of plant proteins. A plethora of information is currently available regarding how the structure of cloned K channels native to animal membranes is related to their function. Therefore, the well-developed model of animal K channel molecular structure will be used as a basis to characterize how ion conductance facilitated by plant K channels is affected by their structure. This presentation should leave the reader confronted by the double-edged conundrum endemic to biological research: The more we learn about a particular biological system, the more we know we have yet to unravel; and, it is often the case that the magnificent elegance and complexity of nature are reflected in the conservation of "simple" solutions which have evolved to solve a particular biological challenge.

Basic Concepts of Membrane Transport

Ions and Membranes

Like all other nutrients which are taken up by plants from the soil solution and which must be transported across both cell and organelle membranes, K is ionic and exists in solution as a charged particle. Potassium, as is the case with all ions, is solubilized in an aqueous solvent (i.e. the soil solution or cell cytosol) by hydrogen bonding of water molecules acting as dipoles. Concentric layers of water molecules (referred to as the sphere of hydration) surround the cation. The partial negative charges at the oxygen end of water molecules align around the cation in the first layer. The second and subsequent shells of water molecules align with the partial positive charges at the hydrogens forming hydrogen bonds with the partial negative charges at the oxygens of water molecules in an adjacent shell. Models of nutrient uptake across plant membranes originally speculated that charged particles such as inorganic ions (a) cannot traverse the hydrophobic, lipid portion of membranes, and (b) move across cell membranes in a hydrated state, i.e., along with their shell of water molecules. Ion channels are one class of proteins which fulfill the first part of this model in that they provide a hydrophilic conduction pathway for ions to traverse the lipid bilayer. We now know the latter part of this axiom to be incorrect; the layers of water molecules are "stripped" off the ion and are replaced by the charged side-chains of specific amino acid residues which line the ion-conducting pathway of the channel protein. Thus, one of the conserved, fundamental features of all ion channel proteins is the amphoteric nature of their structure: They have a hydrophobic (outer) portion which allows them to integrate into the lipid bilayer of a membrane and a (inner) portion of the protein which forms a hydrophilic pore as an ion-conducting pathway.

Electrochemical Gradient

In addition to determining the nature of the transmembrane conduction pathway, the fact that K is a charged particle in solution also impacts the forces which drive flux of this cation across membranes. The driving force for K movement is the electrochemical gradient which exists across a particular membrane. The "chemical" component of this driving force is the difference in K concentration on either side of the membrane. The electrical component of the driving force for K flux is the difference in voltage across the membrane, or membrane potential (Em). The action of the plasmalemma H-pump ATPase dominates the electrical properties of plant cell membranes[36]. Proton pumping out of the cell by this enzyme results in a strongly negative (~ -120 to -200 mV) cytosolic voltage with respect to the cell exterior[27]. This Em drives K uptake by the plant cell against a concentration gradient. The Nernst equation tells us that an Em of this magnitude could allow for passive accumulation of K moving into cells through K channels to a level two orders of magnitude greater than external [K]. In fact, cytosolic K is typically 100-150 mM in plant cells while K in the soil solution is often < 1 mM[5]. Potassium channel proteins act as passive conduits for K flux across membranes; it is the electrochemical driving force across the membrane which will determine whether, and

in which direction, K will move. Actually, K channels function in a far more regulated fashion than simple "pores" in a membrane. Various parts of the K channel protein act in specific manners to facilitate *regulated*, albeit passive, flux across a particular membrane. With respect to voltage-gated channels, the membrane potential has a number of effects on ion conductance through these transport proteins. As mentioned above, the membrane potential is one component of the total force which drives passive flux through the ion-conducting pathway formed by these channel proteins. However, the transmembrane voltage differential also facilitates gating of this conductance. The electric field across the membrane facilitates movement of portions of these proteins (the voltage sensing region), which then results in the opening of the pore, i.e., an increase in conductance. Other portions of these channels also respond to transmembrane voltage by changing the conformation of the protein such that (eventually) an imposed membrane potential can also result in spontaneous closing of the conductance pathway.

Potassium Channel Structure: Pore-forming Subunits

Overview

The following information is summarized from a number of recent reviews of K channel structure[6, 9, 18, 33]. More detailed information about the selectivity, rectification, and gating of K channels is presented in Chapter 5. Potassium channel proteins can be categorized as either voltage- or ligand-gated. Most of the K channels (and all of the plant K channels) cloned so far are voltage-gated. Electrophysiological studies have documented the presence of both inward-, and outward-rectified, voltage-gated K channels in the plasmalemma of a range of plant species and cell types[33]. However, cDNAs encoding only inward-rectifying voltage-gated plant K channels have been cloned to date. We therefore can presently formulate a structural picture of only this class of plant K channels. This review will, consequently, focus on this group of transport proteins.

The first (animal) K channel was cloned in 1988 by extensive genetic analysis and chromosome mapping of a *Drosophila* mutant with a phenotype displaying uncontrolled shaking[30]. The translation product of the gene containing the mutation was deduced to have homology to the known primary structure of cloned Ca and Na channel proteins. Expression of this 'Shaker' gene in a heterologous system resulted in the induction of K-selective currents which had biochemical properties similar to currents through K channels in native *Drosophila* membranes[42]. The observation that expression of the Shaker gene was sufficient alone to result in functional K channels led to the supposition that the Shaker polypeptide formed a transmembrane, ion-conducting K-selective channel protein. Subsequent work led to the demonstration that a functional K channel protein can be formed by the assembly of four of these Shaker polypeptides[18]. Several dozen Shaker homologs have been cloned from a variety of animal species.

Pore-forming Subunits

'Shaker'-type animal K channel polypeptides share a similar tertiary structure, as detailed in chapter 5. These polypeptides have six membrane-spanning regions (S1-S6). One of these transmembrane regions (S4) is a 'voltage-sensor'. It is currently thought that the positively-charged amino acid residues on this transmembrane portion of the polypeptide move (within the membrane) in response to an imposed transmembrane electrical gradient. Movement of this section of the polypeptide facilitates voltage-gating of the currents through the pore. In the case of animal K channels, movement of the voltage-sensor causes the pore to close at hyperpolarizing (i.e., a change from a negative resting potential to a more strongly negative Em) voltages. This results in outward rectification of currents through the channel. At membrane potentials which would drive inward currents (i.e., hyperpolarization would increase the electrical component of the force driving influx of the cation), the voltage sensor closes the channel. A conserved region of Shaker polypeptides, between S5 and S6 (the 'P' region) forms the pore of the channel. It is currently thought that the ion-conducting pathway of K channels is formed by the alignment of the P regions of four K channel (Shaker-type) polypeptides along a central axis perpendicular to the plane of the membrane[12]. The most highly conserved region of K channel pore-forming poly-peptides is within the P domain and includes the nine amino acids (TMTTVGYGD) which have been highlighted as the 'signature sequence' of K channels[15]. It is this stretch of amino acids which forms the inner vestibule of the pore and confers selectivity to the ion currents through the channel. This signature peptide is present (with one or two conservative substitutions) in the sequences of all plant K channels published to date.

An intriguing anomaly in the conservation of structure between cloned animal Shaker polypeptides and plant K channels cloned to date lies in the S4 region. The S4 regions of cloned plant K channel pore-forming polypeptides share significant primary and secondary structural homology to animal Shaker polypeptides. However, the plant homologs of Shaker-type animal K channels are *inward rectifying*, while the Shaker-type animal channels are *outward rectifying*. Expression studies with chimeric channel complexes[7] have shown that the S4 voltage sensor of plant inward rectifying channels likely acts in a similar fashion as the S4 region of animal Shaker channels, but may move in an opposite direction in response to imposed voltage gradients, hence the resulting reversal of current rectification.

Potassium Channel Complexes Contain More than One Type of Subunit

Introduction

Most of what is currently known about the molecular structure of K channel proteins has been formulated from analysis of deduced amino acid sequences as determined from the nucleotide sequences of cloned K channel cDNAs. Study of native K channel proteins is a daunting task, primarily due to the fact that channels are among the lowest in abundance of all known classes of enzymes. It should be remembered that channel proteins are, in fact, enzymes; their catalytic function is the regulated conductance of ions across a membrane. In order to carry out this enzymatic activity, channels move from a closed to an open state. Passive flux of ions, stripped of their shells of hydration through the ion conducting pathway or pore of the channel protein, corresponds to the activity of this class of enzymes. The rate at which ions can flow through the pore of channel proteins is typically orders of magnitude greater than the catalytic rate of most enzymes. Thus, only a few channel proteins need to be present in a native membrane in order to facilitate regulated conductance of a specific ion across this barrier. The high 'enzymatic' activity of channel proteins, then, typically results in their extremely low copy number in a given native membrane system. This characteristic of ion channels has made work with native ion channel proteins technically challenging.

Purification of a Potassium Channel

The first ion channel purified from any native membrane was the Na channel. Purification of this protein was accomplished by using a radiolabeled neurotoxin which bound tightly, and specifically to Na channels, and by using the electricity-generating organ of the electric eel (which is ~70 percent by weight channel protein!) as a source of the target channel[1]. More recently, a similar strategy was followed by two different research groups[29, 31] to successfully undertake the first purification of a native K channel protein. The mamba snake venom polypeptide dendrotoxin was radiolabelled and used to follow the target K channel protein during purification steps, and mammalian brain cortex synaptosomes (enriched in channels) were used as a protein source. As expected, the purification protocol yielded a polypeptide which had a similar size as known Shaker-type K channel polypeptides, and N-terminal peptide sequencing revealed homology to corresponding portions of known Shaker channels, confirming the identity of the purified protein as a pore-forming, K channel polypeptide[35]. The purification protocol used in this initial work with native K channel proteins also identified a second smaller molecular weight polypeptide which at first was thought to be a contaminant. However, subsequent peptide sequencing of the low molecular weight "contaminant" allowed for sequence-specific oligonucleotides to be generated. PCR and subsequent expression library screening yielded a full-length cDNA, encoding this low molecular weight polypeptide which co-purified with the K channel pore-forming subunit[34].

Evidence for the Presence of a β Subunit in Potassium Channels

One of the more powerful techniques used in the study of ion channels is the functional expression of their cloned cDNAs in a heterologous expression system such as the *Xenopus laevis* (South African clawed frog) oocyte. The oocyte is a convenient expression system because it has low endogenous ion channel activity. Potassium channels can be expressed and the induced currents studied without the confounding influence of background K currents.

An important step in our understanding of the molecular structure of K channels occurred when the cDNAs encoding the two polypeptides purified from native brain synaptasome membranes were coexpressed in oocytes. Coexpression of a cDNA encoding the low molecular weight polypeptide along with the cDNA encoding the pore-forming, Shaker-type subunit altered the gating properties of the induced currents[32]. These results demonstrated for the first time that (at least some) native K channel proteins are composed of two different types of subunits; an α subunit which, as described earlier, forms the selective ion conducting pathway across membranes and α β subunit which (in some cases) was shown to alter gating properties of currents through the ion conducting portion of the channel formed by the α subunits.

Characterization of β Subunit Structure and Function

The forementioned preliminary work characterizing (animal) K channel β subunits provided the following details of their structural and functional contribution to K channel proteins. Native K channel proteins are currently thought to be composed of four α (~ 70-80 kD) and four β (~ 30-40 kD) subunits[28]. The β subunit polypeptides are hydrophilic and have putative phosphorylation cites; each β subunit is therefore thought to be cytoplasmic, and bind to the portion of an α subunit protruding into the interior of the cell[11]. Not much is known just yet about how the β subunit physically interacts with the K channel α subunit. Some speculations in this area have been made based on sequence analysis of cloned β subunit polypeptides. It has been suggested[23] that β subunits may affect α subunits, and exert an effect on K channel activity, through an oxidoreductase function. They may use cytosolic reductant (NADH) to reduce amino acid residues on the α subunit. The basis for this hypothesis is as follows. Chouinard et al.[10] and McCormack and McCormack[23] have noted that K^+ channel β subunits share some sequence homology with members of a superfamily of enzymes that

utilize reduced pyridine nucleotide as a cofactor and catalyze a broad range (i.e., with regard to substrate) of oxidoreductase reactions. Members of this enzyme superfamily are functionally quite diverse; nonetheless, they demonstrate substantial conservation of primary sequence related to the presence of secondary structural elements in these proteins. Specifically, amino acid motifs corresponding to the β sheets present in the α/β barrels formed as part of the tertiary structure of aldo-keto reductases are conserved[10]. Potassium channel β subunits demonstrate sequence homology to these regions of the oxidoreductase enzymes. Attributing an oxidoreductase function to β subunits remains speculative. This enzymatic activity has not yet been demonstrated in preparations of either purified native or *in vitro* translated β subunit polypeptide.

This oligomeric structure of a K channel protein complex is represented in **Figure 1**. It is not yet clear whether only some, most, or all native K channel proteins share this oligomeric structure and are comprised of two different types (i.e. α and β) of subunits. Recent studies with native K channel protein complexes in animal cell membranes as reviewed by Catterall[9] and *in situ* RNA hybridization studies with mammalian brain tissue (D. Parcej, personnel communication) indicate that β subunits may be components of all K channels and are ubiquitous in animal cell membranes. As will be discussed later in this chapter, work from our laboratory with *Arabidopsis thaliana* cell membranes suggests that this may also be the case in plants.

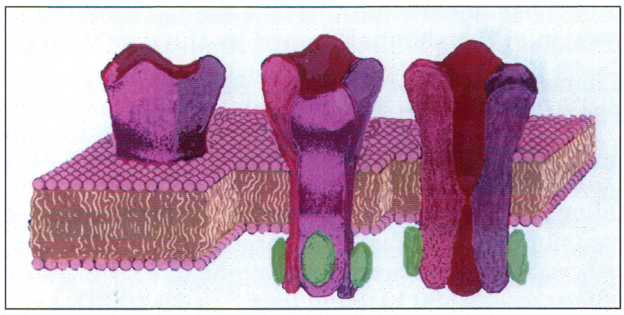

Figure 1. Model of the three-dimensional structure of K channel proteins, shown traversing a cell membrane. In the center of the figure, a K channel complex is shown with four membrane-spanning α subunits, with a β subunit bound to the cytoplasmic portion of each α polypeptide. On the right, the channel is portrayed with the front half removed, revealing the pore formed by the alignment of the α subunit P regions.

The initial functional characterization of the β subunit[24, 32] indicated that the presence of this polypeptide in K channel protein complexes affected (voltage-dependent) inactivation of currents. As compared to channels formed from only four α subunits, addition of the β subunit transformed some non-inactivating K channels into fast-inactivating types and increased the inactivation rate of K channels formed from other α subunits. Although most of these functional studies have reported effects of the β subunit on inactivation kinetics of channels[10, 21, 24, 32], there is one report of a β subunit altering the voltage dependence (i.e. voltage at which current flows through the channel) and kinetics of voltage-dependent activation of K channels[10]. At this point, cDNAs encoding several different animal β subunits have been cloned. It should be pointed out that some of these cloned β subunits have failed to induce inactivation of currents through the part of the channel formed by the four α subunits in coexpression studies[21, 24, 32]. The function of these β subunits is still a subject of active research. More recent studies[14, 37] suggest a different, and more fundamental role that these β subunits may play in K channel function. This work suggests that β subunits may act as chaperone-like polypeptides and play a role in biosynthetic maturation and surface expression of the holoenzyme K channel complex. This latter putative role of β subunits may be more relevant to the situation in plants. Most native plant K channels display non-inactivating voltage-dependent currents. The function some of the animal β subunits play in facilitating inactivation of the channel may not be physiologically relevant to the role K channels play in plant cell membranes. For a more thorough discussion of this topic, readers are referred to chapter 5, and to a recent review by Schroeder et al.[33].

Ball and Chain Inactivation

Gating mechanisms are typically critical to the physiological function of ion channels in animal cell membranes because channels contribute to the generation of action potentials of excitable cells. A combination of currents (usually Na and K) results in the rapid depolarization and then repolarization of the cell membrane potential over the course of several milliseconds in these excitable cells. The action potential is generated by the specific opening, then rapid closing of ion channels. The closing of the channels occurs in response to the rapid changes in Em which occur during the action potential (i.e. the channels are voltage gated, and as the Em changes, the percentage of channels which are open changes). However, channels which are open at a given membrane potential close over time (i.e. resulting in the maintenance of a fully open state and maximal conductance for a brief, ~ millisecond duration), even if membrane potential is held constant. At a given Em, currents through (many) animal K channels demonstrate rapid time-dependent inactivation (the conductance through the channel decreases). As mentioned above, the role K channels play in plant physiological function typically does not warrant such rapid changes in conductance. Most K currents recorded from native plant membranes demonstrate only comparatively slow, or no inactivation.

Although there are some other mechanisms which facilitate inactivation of currents through

animal K channels, the most common means by which conductance is decreased through the ion-conducting pathway formed by the four α subunits is by N-terminal "ball and chain" inactivation. The following information detailing this system of channel gating is summarized from the review presented by Jan and Jan[18].

Nitrogen-terminal inactivation of currents through the ion-conducting pathway is facilitated by the voltage-dependent movement of a portion of the channel protein into the inner mouth of the channel pore, occluding the conductance pathway. The portion of the protein which physically occludes the pore is envisioned to have the tertiary structure of a ball. The (overall) hydrophilic ball structure is known to be present on the portion of the K channel protein which protrudes into the interior of the cell. The ball structure is "tethered" to the rest of the channel protein by a "chain" formed by hydrophilic amino acids which have little constraint imposed on their movement by the structure of the rest of the protein. This inactivation ball is thought to be formed by the first (N-terminal) ~ 20 amino acids of the α subunit in the case of some specific animal K channel proteins. Not all animal K channel α subunits are inactivated by an N-terminal ball-and-chain structural component of the α subunit. A similar length of amino acids at the N-terminus of some (but not all) animal β subunits can also form this ball structure. Current models of K channel structure suggest that the presence of this N-terminal ball structure in some β subunits is thought to confer the ability of these polypeptides to affect the gating properties of K channel proteins of which they are a component.

Different K channel β subunits are expressed from a single locus by alternative splicing. The β subunits cloned from animals to date can be categorized from analysis of their deduced sequences into three different splice variants. These polypeptides all share a highly conserved central core region and differ primarily with regard to the varying length of their N-terminal extensions. Animal β subunits with relatively long N-terminal extensions include regions which form the ball and chain, conferring on them the ability to facilitate gating of the K channel protein. Those that lack this extension have no effect on gating properties but have been demonstrated to enhance surface expression of the K channel complex.

β Subunits in Plant Potassium Channels

Work from this laboratory[13, 39, 40, 41] led to the cloning of the first two plant K channel β subunits, KAB1 and KOB1 from *Arabidopsis thaliana* and rice, respectively. These β subunits share 45-50 percent overall amino acid identity with animal K channel β subunits, but show a higher degree of sequence conservation in regions corresponding to the putative oxidoreductase functional domains. Based on the forementioned analysis of animal β subunit structure:function, both KAB1 and KOB1 can be classified as "short" β subunit polypeptides, lacking the N-terminal extension forming the inactivation ball of longer animal β subunits. As shown in **Figure 2**, the N-terminal region of KAB1 (and KOB1, not shown) is shorter than the shortest animal β subunit cloned to date. The N-terminus of the plant β subunit polypeptides represents the beginning of the "core" region shared by all of the (animal) β subunit splice variants.

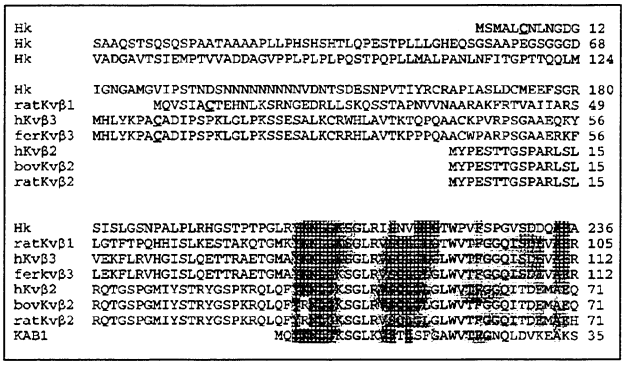

Figure 2. Alignment of the N-termini of (deduced) K channel β subunit amino acid sequences. Numbers on the right refer to the position in each sequence of the amino acid directly to the left. The β subunit sequences shown in this figure are the *Drosophila* polypeptide from the hyperkinetic locus (Hk), the β1 (ratKvβ1) and β2 (ratKvβ2) polypeptides from rat brain, the β2 (hKvβ2) and β3 (hKvβ3) polypeptides from human atrium, the β2 polypeptide from bovine brain (bovKvβ2), and KAB1 from *Arabidopsis thaliana*. A cysteine (C) residue is present near the N-terminus of Hk, ratKvβ1, hKvβ3, and ferKvβ3, while this amino acid residue is absent from the shorter N-termini of the β subunits (hKvβ2, bovKvβ2, ratKvβ2, and KAB1) which have not been found to affect gating of currents through α subunits. This cysteine residue is thought to be involved in β subunit binding to the α subunit in K channel proteins which show β subunit inactivation (see ref. 40 for further discussion). Amino acid identity in the beginning of the central core region of the β subunit sequences is portrayed by shading.

We have examined the effect KAB1 coexpression has on plant K channel α subunits (X. Zhang and G. Berkowitz, unpublished data). Coexpression (in frog oocytes) of KAB1 with the plant K channel α subunit KAT1 did not alter any gating parameters of the induced currents. However, at a given hyperpolarizing voltage, whole-cell (inward) K currents were increased in the presence of KAB1. We interpret this result as consistent with the possible role β subunits have on assembly and expression of the channel complex. Greater whole-cell currents would result from an increase in the level of functional expression of the channel complex. Thus, plant K channel β subunits such as KAB1 may enhance the stability of the oligomeric channel complex during expression and insertion into membranes.

Results from a series of experiments we have recently undertaken (J. Ma, X. Zhang, G.

Berkowitz, unpublished data) offer a final, intriguing point regarding gating and inactivation of currents through plant K channel α subunits. As discussed above, native plant K channels do not demonstrate inactivation gating typically found in animal (voltage gated) K channels. We also are unaware of any cloned plant α subunit which displays inactivating currents upon functional expression or any plant β subunit which confers inactivation upon coexpression with α subunits. These findings suggest that inward rectifying voltage-gated plant K channels are incapable of inactivation. In order to evaluate the capacity of plant α subunits to undergo inactivation, we generated a chimeric construct of the plant K channel α subunit KAT1 which contained a portion (the NAB domain, see below) of the N-terminus of a Shaker-type animal K channel, Kv1.4. The animal α subunit Kv1.4 is capable of facilitating N-terminal inactivation. It contains the N-terminal "ball and chain" sequence. Generating the KAT1 polypeptide with the NAB domain allowed for co-assembly of heteromeric channels containing plant (i.e. KAT1 with the added NAB domain) and animal (Kv1.4) α subunits. Functional expression of these heteromeric channels in oocytes resulted in inward K currents (i.e. currents through channel complexes containing KAT1). However, these inward currents underwent fast inactivation, presumably due to the ball of a Kv1.4 peptide occluding the pore of the heteromeric channel. These results suggest that plant inward rectifying K channel α subunits are capable of inactivation, if a ball portion of the protein is present to bind to the inner vestibule of the pore.

The Molecular Structure and Assembly of Plant Potassium Channels

α:α Subunit Binding

Animal outward rectified (i.e. 'Shaker'-type) K channels can be divided into four subfamilies; Shaker (Kv1), Shab (Kv2), Shaw (Kv3), and Shal (Kv4)[6, 9, 18, 30]. Heteromeric complexes can form between α subunits within each of these families, but not across these subgroupings of channels[43]. Protein:protein interaction studies have demonstrated that a conserved region in the N-terminus of these polypeptides, referred to as the NAB domain, is responsible for the promotion of α:α subunit binding and for preventing these associations between α subunits of different subfamilies[38, 43, 44]. The NAB domain is apparently not present in the N-terminus of plant K channel α subunits; the basis for α:α subunit binding in plants has not yet been characterized in the published literature. However, sequence analysis has led us to an intriguing speculation regarding the structural basis for plant K channel α:α subunit associations.

Our speculations regarding plant K channel α:α subunit interactions began with a homology analysis (**Figure 3**) of cloned plant (**Figure 3A**) and animal (**Figure 3B**) K channel α subunits. The results of such an analysis indicate that, as discussed previously, there is a high degree of primary sequence conservation in regions of these polypeptides which are known to be involved with various aspects of K channel function. In both plant and animal K channel α subunit polypeptides, the pore region and the membrane spanning regions (S1-S6, including the voltage sensor S4) demonstrate relatively high degrees of sequence conservation. As shown in **Figure 3B**, animal K channel α subunits have a long (mostly hydrophilic) N-terminal region prior to the conserved, membrane spanning (S1-S6) sequences. The only conserved portion of

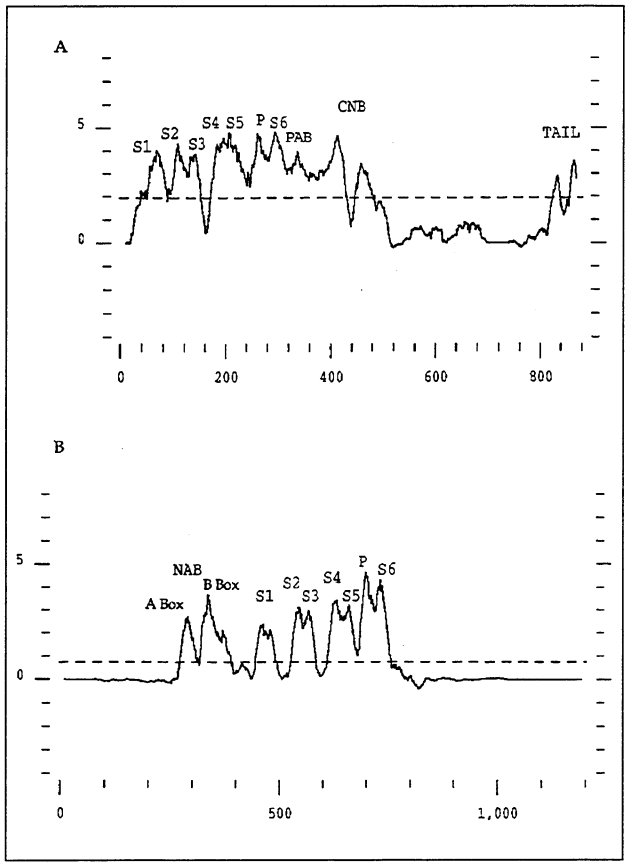

Figure 3. Similarity plots of (A) the plant K⁺ channel a subunits KAT1, AKT1, AKT3, and KST1, and (B) for eight animal Shaker-related K⁺ channel a subunits. These similarity analyses were generated using GCG (Genetics Computer Group, Madison, WI) software; PILEUP program for sequence alignment followed by PLOTSIMILARITY to generate plots of sequence similarity. The numbers on the horizontal axis represent relative position in the alignment, and do not correspond to the actual amino acid residue position in an individual sequence. The vertical axis represents relative similarity in arbitrary units. The heights of the peaks are indicative of the relative degree of sequence conservation (higher values indicate greater conservation). Average similarity for the sequences plotted in each analysis is portrayed by the broken line. In addition to the membrane spanning regions of the proteins (S1 through S6 and the P region), the "PAB" box, cyclic-nucleotide binding (CNB) domain, and tail region of plant polypeptides are indicated, and the A box and B box of the NAB domain of animal polypeptides are indicated.

this N-terminal leader sequence is the NAB domain, which is made up of a conserved "A Box" and "B Box"[44]. Plant K channels, in contrast, have a very short N-terminal sequence preceding the S1-S6 domains (**Figure 3A**). This short N-terminal region of plant K channel α subunits has a relatively low degree of sequence conservation and does not contain regions homologous to the NAB domain found in animal channels. In contrast to the short N-terminus of plant α subunits, the (hydrophilic) C-terminal region of plant α subunits extending beyond the last membrane spanning sequence (S6) is quite long. This extended C-terminus of plant α subunits shows little sequence conservation with the exception of a region we denote as a "PAB" box (see discussion below) next to the S6 sequence, a putative cyclic nucleotide binding domain (discussed in ref. 33), and a region we refer to as the "tail" at the extreme C-terminus of the polypeptide (**Figure 3A**). These regions of sequence conservation in plant α subunits in the portion of the polypeptide C-terminal to the S6 sequence contrast with the low degree of homology in the C-terminus of animal α subunits, as shown in **Figure 3**. Intriguingly, we have noted some sequence indentity between the PAB box ('**P**lant **A** and **B** box') of plant K channel α subunits which is towards the C-terminus of the (S1-S6) membrane-spanning regions and the A and B boxes of the NAB domain of animal K channel α subunits, which is present at an entirely different portion of the polypeptides, N-terminal to the (S1-S6) membrane-spanning regions. The comparison shown in **Figure 4** identifies regions of the PAB domain of the plant α subunit KAT1 which have some sequence identity (29 percent and 38 percent, respectively) to the A and B boxes of the NAB domain in the N-terminus of animal α subunits. We also note some homology in secondary structure amongst these regions of the polypeptides (**Figure 4**). This analysis led us to speculate that regions C-terminal to S1-S6 (specifically the PAB box) of plant α subunits may play a role in α:α subunit binding and channel assembly. The identification of a region towards the C-terminus of plant α subunits as critical for subunit recognition and channel assembly would be in marked contrast to mechanisms facilitating α:α subunit interactions in animal K channels. Research from many different labs using a variety of approaches has demonstrated that α:α and α:β interactions and assembly of functional animal K channels are dependent on the α subunit N-terminus (including regions around the NAB domain), and that the C-terminal portion of the α subunit polypeptide is not required for channel assembly[2, 16, 19, 20, 38, 44].

Results of current research in our laboratory (J. Ma and G. Berkowitz, unpublished data) are consistent with a role for the plant α subunit C-terminus (including the PAB box) in subunit binding, assembly of the channel complex, and functional expression of plant K channels. A summary of the

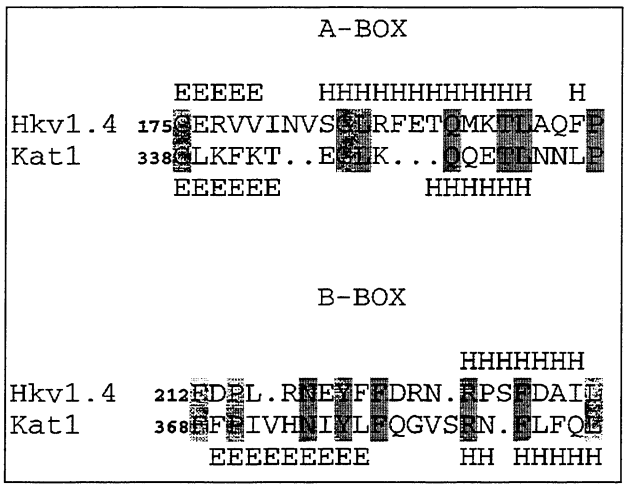

Figure 4. Alignment of the C-terminal tail region of the plant α subunit KAT1 with the A box and B box of the N-terminal NAB domain of the animal K channel α subunit Hkv1.4; deduced amino acid sequences are shown using single letter abbreviations. Numbers on the left of the sequences correspond to the position of the amino acid directly to the right of the number. Predicted secondary protein structure is portrayed on the top for the Hkv1.4 sequence and on the bottom of the KAT1 sequence. Secondary protein structure was developed using the PHDsec program (available from the EMBL WWW server: www.embl-heidelberg.de/predictprotein.html). H denotes predicted helix structure, E denotes β strand, and blank regions denote no regular structure, or presumed loops. Regions of primary sequence identity are shaded.

experimental evidence supporting this conclusion is as follows. Deletion of the first (i.e. N-terminal) 28 amino acids of KAT1 did not affect functional expression in oocytes. These results suggest that in plant α subunits, a significant portion of the total N-terminal region upstream from the first membrane spanning region (i.e. S1 starts at L66) is not required for functional assembly of plant α subunits. (Marten and Hoshi[22] published similar results.) A significant portion of the KAT1 C-terminus can be deleted (D541-677, i.e. the C-terminal 137 amino acids) without effects on functional expression. Apparently, the most extreme portion of the C-terminus of plant α subunits, including the conserved tail region, is not critical for functional channel expression. However, deletion of a C-terminal portion of KAT1 which includes the PAB box prevents functional expression. We further investigated the effect the PAB box of KAT1 has on subunit assembly using assays of protein:protein interaction (methods described in ref. 40). We found that a C-terminal portion of KAT1 (amino acids 308-677) which includes the PAB box was able to bind to the full-length KAT1 polypeptide *in vitro*. We also determined that the KAT1 N-terminus (amino acids 1-54) is able to bind full-length KAT1. These results together support a model of plant K channel α:α subunit interaction which is quite different than our current understanding of the basis for α subunit assembly in animal K channels.

We currently speculate that instead of the N-terminal NAB domain, the C-terminal PAB box is critical for α subunit interactions and channel assembly. However, our current model includes involvement of the N-terminus in α subunit interactions. We believe that the N-terminus of one α subunit can physically interact, and bind with the C-terminus of another plant K channel α subunit. These α subunit interactions are portrayed as part of the physical model of plant K channel assembly shown in **Figure 5**.

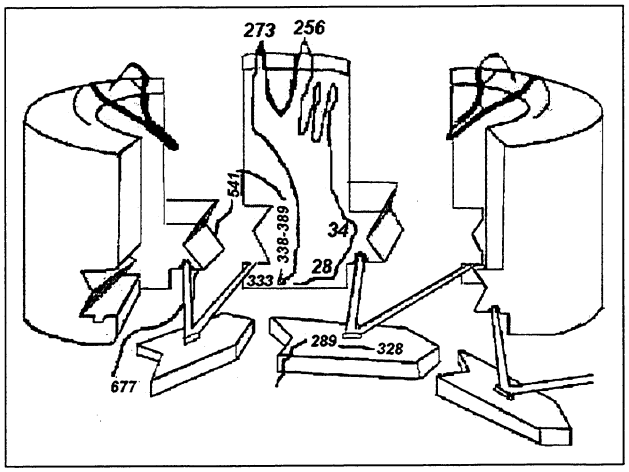

Figure 5. **Model of protein:protein interactions between plant K channel subunits. Three α subunits are shown at the top of the diagram, and three β subunits (arrow structures) are shown at the bottom. The P, or pore region of the a polypeptides, is portrayed as a hairpin loop (formed by amino acids 256 through 273) at the top of each subunit. For the α subunit in the center of the diagram, the entire primary sequence is portrayed as an unbroken line. This model portrays regions of binding between both α and β subunits. The β subunits are portrayed as binding to other β subunits adjacent in the protein complex, and also to the N terminal region (near amino acid 34) of one α subunit and the C terminal region (after amino acid 333) of an adjacent α subunit. The portion of the β subunit binding to α polypeptides is portrayed as including amino acids 289-328 in the b polypeptide.**

Involvement of the β Subunit in Plant K Channel Assembly

Our demonstration that coexpression of KAB1 with KAT1 increases whole cell currents in oocytes, as discussed previously, is suggestive of a role for β subunits in efficient assembly and expression of the plant K channel protein complex. Further studies of protein:protein interactions between plant K channel subunits (J. Ma and G. Berkowitz, unpublished data) support this contention. We found that the plant β subunit KAB1 bound to both the N-terminus (amino acids 1-54) and the C-terminus (amino acids 333-677) of the α subunit KAT1. We localized one specific region of the β subunit responsible for these physical associations as the C-terminus (amino acids 289-328) of the KAB1 sequence. Deletion of this region of the β subunit prevented binding to both the C-terminal and N-terminal regions of plant α subunits. The model of physical interaction between plant K channel subunits shown in **Figure 5** incorporates these results; a region of the β subunit including amino acids 289-328 is portrayed as interacting with the N-terminus of one and the C-terminus of another α subunit. Our protein:protein interaction studies also demonstrated a physical interaction *in vitro* between plant β subunits. We used the yeast two-hybrid system[3] to investigate the *in vivo* physical interactions between plant K channel β subunits. Results from the yeast two-hybrid system demonstrated that the physical interaction between plant β subunits (i.e. between KAB1 and KAB1) could be prevented if one of the constructs had either the first 28 (D1-28), or the last 39 (D289-328) amino acids deleted from the KAB1 sequence. Collectively, the results of our functional expression, and protein:protein interaction studies suggest the following model (portrayed in **Figure 5**) of plant K channel subunit interaction and protein assembly. The C-terminus of one β subunit binds to the N-terminus of an adjacent β polypeptide. A region of the β polypeptide including amino acids 289-328 can bind to the N-terminal region of one α subunit and the PAB box region towards the C-terminus of an adjacent α polypeptide. The same two regions of adjacent α subunits also physically interact. The presence of (presumably) four β subunits, interacting with each other and with adjacent α subunits, stabilizes the assembly of the channel complex.

Expression of Plant K Channel Subunits *in Situ*

Recent studies from a number of different groups have begun to provide us with a better understanding of the expression patterns of plant K channel polypeptides. The *A. thaliana* K channel α subunit KAT1 is expressed primarily in leaves, specifically in guard cells[26], while the α subunit AKT1 is primarily expressed in roots[4] of the same plant species. The KAT1 subunit is likely involved in K movements facilitating turgor changes in guard cells, while AKT1 plays a role in passive uptake of K into plants. The AKT2, cloned from the same plant species, is expressed in leaves, but this α subunit has not yet been functionally characterized[8]. KST1, a homolog of KAT1 cloned from potato, is also likely expressed in guard cells of leaves[25]. The *A. thaliana* β subunit KAB1 is expressed in leaves, flowers and roots[40]. Since the α subunits KAT1 and AKT1 are expressed in leaves and roots, respectively, and KAB1 expression is ubiquitous, we speculate that *in vivo*, the β subunit KAB1 can bind to different α subunits. Our *in vitro* binding studies support this assertion; KAB1 was found to bind to both KAT1 and AKT1, but not an animal K channel α subunit (J. Ma and G. Berkowitz, unpublished data). Homologs of KAB1 have been identified in seven of 15 plant species tested[39]. Expression of the KAB1 homolog in broad bean has been shown to be ~ 80-fold greater in guard cells than in meso-

phyll cells[40]. The KAB1 homolog in rice, named KOB1, has been cloned[13]; it is expressed in virtually all plant parts tested, with highest levels in leaves (especially the flag leaf) as compared to roots, the ear, and seed. Interestingly, the expression of KOB1 was found to be affected by cell K status. Potassium deficiency resulted in down regulation of KOB1 expression. This is the first example known to us of ion channel gene expression being modulated by the specific ion transported by the expressed protein[13]. Cellular K status was found by Basset et al.[4] specifically not to affect expression of AKT1.

The KAB1 antibody has been used in our laboratory to facilitate the first (subcellular) immunocytochemical localization of a plant K channel[39]. Immunogold staining detected KAB1 protein in the chloroplast inner envelope, mitochondria, tonoplast, and plasmalemma. The protein was evident as discreet, single K channel complexes regularly spaced in these membrane systems (an example is shown in **Figure 6A**). The regular pattern of K channel expression in these membrane systems allowed for a structural estimation of K channel density in plant cell membranes. An example of the predicted expression pattern of individual K channel protein complexes in the plasmalemma is shown in the model cell portrayed in **Figure 6B**. This analysis confirms the prediction that K channel proteins are expressed in extremely low copy number in most membrane systems.

The results presented in this chapter should lead the reader to the realization that the molecular analysis of plant K channel proteins is just beginning. Much work is still ahead before our understanding of this important class of plant transport proteins is at a level which will facilitate the genetic engineering of crop plants which demonstrate enhanced phenotypes which would be dependent on altered patterns of cation uptake.

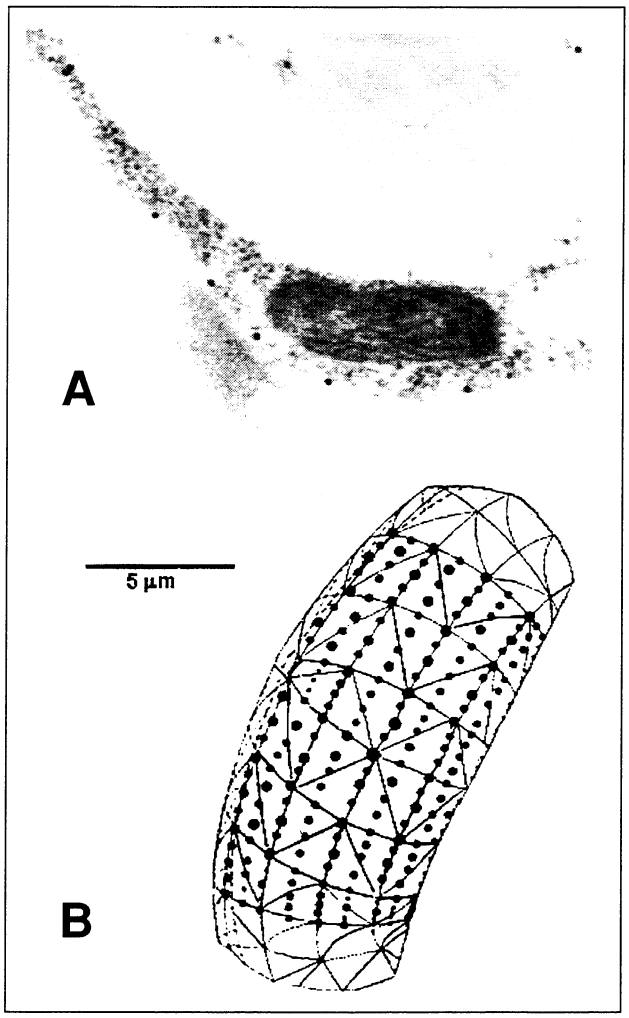

Figure 6. Deduced K channel protein density in the plasmalemma of a model plant cell; (A) Electron micrograph showing the regular pattern of immunogold staining (~ 0.25 µm between each protein complex) along the plasmalemma using the KAB1 antibody and immunogold staining; (B) A model of a portion of the surface of a plant mesophyll cell, showing the deduced density of individual K channel protein complexes along the surface of the plasmalemma.

Addendum

The work described in this chapter was supported by the National Science Foundation under grants BIR-9512977 and MCB-9513921, and the U.S. Department of Energy, Energy Biosciences Program under grant DE-FG02-95ER20202. During the preparation of this text, Czempinski et al. reported the cloning of the first plant outward-rectifying K channel (Czempinski, K., S. Zimmermann, T. Ehrhardt, and B. Muller-Rober, 1997. New structure and function in plant K channels: KCOl, an outward rectifier with a steep Ca^{2+} dependency. EMBO J. 10:2565-2575.) The analysis of the molecular structure of plant K channels presented in this chapter does not include information about this new class of proteins. This chapter is dedicated to the memories of two renowned plant physiologists, colleagues, and friends: Dr. Bruce Wasserman (Rutgers University) and Dr. Richard Crain (University of Connecticut).

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Chapter 4:
Molecular and Physiological Aspects of Potassium Absorption by Higher Plant Roots

Frans J.M. Maathuis

Department of Biology, University of York, PO Box 373, York YO1 5YW, United Kingdom

Introduction

Plant growth, ultimately fueled by solar energy, critically depends on a sufficient uptake of inorganic nutrients. Especially in crop plants, it is of utmost importance to understand the principles behind absorption of nutrients in order to rationalize and optimize nutrient delivery. Potassium as a macronutrient and a major constituent of fertilizers is a case in point. In this chapter I will describe how recent research contributed to our understanding of processes involved in K^+ absorption occurring at the root-soil interface.

The strictly regulated level of cytoplasmic K^+ at around 100 mM in both animal and plant cells points to its crucial role in cell homeostasis. In plants, the functions of K^+ have been amply described (e.g. 28 and references therein and this volume) and can roughly be identified as either biophysical or biochemical in nature. As the principal cationic species in the cytosol, K^+ acts as counterion for the large excess of negative charge on proteins, nucleic acids, and organic acids. Furthermore, a wide range of cytosolic reactions is activated by K^+, including some central to intermediary metabolism (e.g. pyruvate formation) and various stages of mRNA translation.

As predominant cation in the vacuole, K^+ contributes extensively to cell turgor and therefore plant rigidity. However, K^+ is not essential to maintain cell turgor, and various other (mono or divalent) cations and/or organic compounds can substitute for K^+. This interchangeability between K^+ and other cations is most strikingly observed in halophytes where most of the vacuolar cation contents will exist in the form of Na^+ rather than K^+.

Maintaining the essential roles of K^+, despite large environmental fluctuations in K^+ supply, requires sophisticated means of K^+ uptake which, in terrestrial plant species, occurs at the interface between root and soil solution (in several aquatic species absorption by shoot tissue may also contribute). Potassium ion concentrations in the soil solution ($[K^+]_{ext}$) can vary from a few μM to tens of mM but will typically range from 100 to 1000 μM, even in fertilized soils[32]. Apart from spatial variation on a large scale, $[K^+]_{ext}$ will also vary extensively over short distances and in time, with soil pH, moisture, and chemical composition all having marked effects[35].

Rb^+ Uptake Studies

The wider availability of radio isotopes in the late 1950s and early 1960s greatly progressed our knowledge of nutrient uptake in plants. Epstein and coworkers used $^{86}Rb^+$ as a K^+ analog and exhaustively characterized Rb^+ uptake in barley roots using external concentrations in the μM and mM range[8]. The observed kinetics with respect to $[K^+]_{ext}$ were interpreted as resulting from two uptake systems working in parallel at the plasma membrane with a high-affinity system operating when $[K^+]_{ext}$ is in the micromolar range and a low-affinity transport system which is dominant at millimolar $[K^+]_{ext}$ (**Figure 1**). High-affinity uptake displays a K_m of 10-40 μM, a relatively high-affinity for Rb^+ or K^+ compared to Na^+. Its function is not affected by the accompanying anion, and the presence of Ca^{2+} is vital to maintain a high degree of selectivity for K^+. The low-affinity mechanism exhibits saturation and a millimolar K_m, although in some cases its dependence on $[K^+]_{ext}$ is linear[17, 18]. It shows a fairly low selectivity for K^+ over other alkali cations and a K^+ transport performance which varies for different accompanying anions.

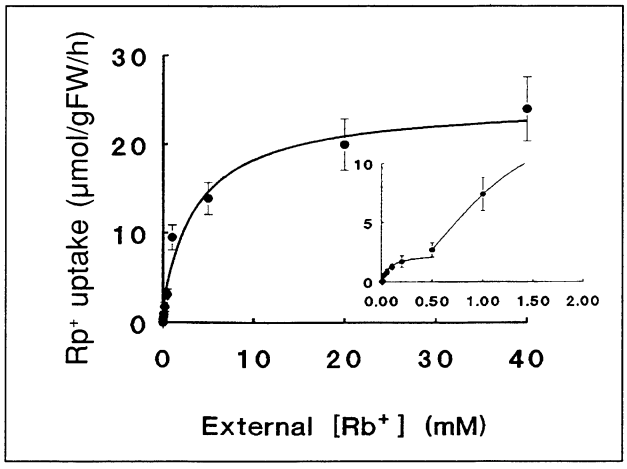

Figure 1: **Biphasic Rb^+ absorption by roots of *A. thaliana* as a function of the external Rb^+ concentration. Low-affinity uptake saturates at Rb^+ concentration >30 mM with an apparent K_m of approximately 5 mM. Uptake mediated by the high-affinity mechanism saturates around an external Rb^+ concentration of 300-500 μM with an apparent K_m of 80 μM. The inset shows the same data for high-affinity uptake on an expanded scale.**

The interpretation of two parallel uptake systems at the plasma membrane was criticized in several reports (see 7 for overview), and alternative explanations for multiphasic kinetics included the contribution of different types of tissue and the involvement of the tonoplast to the overall uptake.

As the number of unidirectional influx studies grew, it became clear that most of these criticisms were unfounded: it was shown that the high-affinity uptake phase was selectively inhibited with sulfhydryl reagents and the low-affinity phase with the K channel blocker tetra ethyl ammonium (TEA) arguing in favour of the existence of two separate mechanisms[17, 18, 21]. Additionally, studies using single cell algae[7] showed that biphasic uptake was based on a cellular level and did not originate in the contribution of various tissues. On the basis of distinct properties such as kinetics, pharmacology, selectivity and specific dependence on the growth medium K^+ status of the different uptake phases, it became therefore generally accepted that the biphasic characteristic of K^+ uptake results from the operation of two independent transport mechanisms.

Thermodynamics of K^+ Uptake

Normally, the K^+ concentration in the cytoplasm ($[K^+]_{cyt}$) is several orders of magnitude higher than the $[K^+]$ at the root surface, and this large (outward) gradient can only be sustained at the expense of constant energy input. Epstein speculated that high-affinity uptake was mediated 'actively' by a 'carrier', implicating the direct dissipation of metabolic energy, whereas low-affinity uptake involved 'passive' transport of K^+ down its electrochemical gradient by 'facilitated diffusion.' [It should be noted that 'passive' transport is by no means free in terms of energy expenditure (see below) since it dissipates the membrane voltage.] Later reports (e.g. 25) similarly concluded that high-affinity K^+ uptake probably required direct energization (e.g. by coupling to other ion gradients or metabolising ATP) and that low-affinity uptake displayed the characteristics of an electrodiffusion process, possibly mediated by ion channels[17, 18]. It was therefore recognized that a fundamental difference between the mode of energizing high and low-affinity uptake was likely.

Transport over the plasma membrane of a charged ion is determined by two parameters: the chemical gradient, i.e., the difference between the external and cytoplasmic concentration and the electric gradient, i.e., the cell membrane voltage. Combined, these two forces are expressed as an electrochemical potential which is either outward or inward directed. For K^+, the chemical gradient will always be outward directed under physiological conditions, whereas the negative value of the membrane voltage constitutes a potential driving force for K^+ uptake against the chemical gradient. However, the extent to which K^+ is accumulated when the membrane voltage is the only driving force will depend on the values of both the membrane voltage (E_m) and the K^+ gradient as expressed by the Nernst equation

$$E_m < 60 \log \frac{[K^+]_{ext}}{[K^+]_{cyt}} \qquad \text{Eq. 1}$$

From this equation it follows that with a typical value for E_m of -120 mV, 'passive' K^+ accumulation which is driven exclusively by E_m is limited to 100 times $[K^+]_{ext}$. Potassium ion accumulation to levels higher than 100 times $[K^+]_{ext}$ therefore requires alternative modes of energization and rely on 'active' transport. Clearly, to adequately assert the competence of E_m to energize K^+ uptake (without the involvement of other energy sources), demands an accurate measurement of the parameters E_m, $[K^+]_{ext}$ and $[K^+]_{cyt}$. Thus, although it is relatively simple to determine the E_m of root cells, values of $[K^+]_{cyt}$ were often estimated and firm conclusions regarding the mode of energisation (e.g. 'passive' versus 'active') of K^+ transport were not possible.

By measuring all the relevant parameters in *Arabidopsis thaliana* roots, it was concluded that E_m driven K^+ accumulation could sustain the observed inward K^+ fluxes and maintain $[K^+]_{cyt}$, as long as $[K^+]_{ext}$ remained higher than approximately 1 mM[25]. Whenever $[K^+]_{ext}$ drops below this value, K^+ accumulation is against its electrochemical gradient and consequently has to be energised in an alternative way. Determination of the same thermodynamic parameters in barley[47] led to a comparable lower limit of $[K^+]_{ext}$ where passive K^+ influx can be sustained. The value of 1 mM agrees well with those obtained in a multitude of Rb^+ flux studies where highs and low-affinity uptakes attains dominance on either side of similar values of $[K^+]_{ext}$ (**Figure 1**). Evidently, this value will depend on an accurate recording of E_m, and some authors have pointed out that the use of impaling microelectrodes may lead to underestimations of E_m, due to the imperfect membrane seal around the electrode tip[12]. A 30 mV error in E_m would lower the value of $[K^+]_{ext}$ where E_m driven uptake is competent from 1 mM to around 0.3 mM.

Low-Affinity K^+ Uptake

Ion Channel Involvement

The capacity of the membrane voltage to drive low-affinity K^+ accumulation does not necessarily imply that such a scheme functions under physiological conditions. Nor does it identify a specific molecular mechanism that mediates 'passive' K^+ uptake. However, since in principle, low-affinity uptake could be E_m driven, and from its sensitivity to the potassium channel blocker TEA, ion channel involvement was inferred[17, 42]. Furthermore, ion channels, which form ion selective pores embedded in the membrane, show high turnover rates of $>10^6$ ions per second which are necessary to carry the large fluxes typical of low affinity K^+ uptake in intact plants. [For a more thorough treatment of plant channel structure function relations see Chapter 5 in this volume].

Plant K^+ Channels

Application of the patch clamp technique revealed that in all investigated plant cells potassium

selective, voltage activated ion channels (K^+ channels) are present and often form the most dominant channel species. K^+ channels are believed to play an important role in the process of K^+ absorption plant movement, turgor regulation and the maintenance of a negative E_m (see Chapter 5 of this volume). K^+ channels belong to either of two broad categories (**Figure 2**): one class tends to open when E_m becomes less negative than the potassium equilibrium voltage (E_{K+}, defined as 60 log $[K^+]_{ext}$ / $[K^+]_{cyt}$ as described in equation 1), it allows an outward K^+ flux and is thus termed an outward rectifying channel (ORC). If E_m becomes more negative than E_{K+}, another class of channels is switched to the open state allowing an inward K^+ flux through so called inward rectifying channels (IRC). Clearly the values of E_m and E_{K+} are crucial in determining which class of channel is activated and to what degree. Furthermore, E_m in combination with E_{K+} determine whether the K^+ flux is directed into or out of the cell and also determine the magnitude of the driving force.

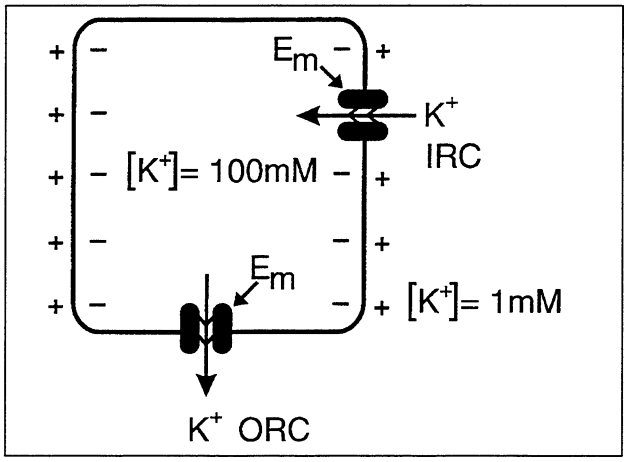

Figure 2. Schematic overview of the two classes of dominant K^+ channel present in the plasma membrane of a typical root cell. Depending on the membrane voltage and the K^+ gradient, K^+ fluxes will be directed outward or inward. Similarly, opening of either ORC or IRC occurs when the membrane voltage is positive (ORC opening) or negative (IRC opening) of the K^+ equilibrium voltage (E_{K+}). Assuming the K^+ activities shown in the figure, IRC opening would take place when the membrane voltage is more negative than -120 mV, whereas ORC open when it is less negative.

To function as a low-affinity K^+ uptake pathway, channels should be gated open when the electrochemical gradient for K^+ is inward and closed if the opposite occurs. *In vivo*, if the cytoplasmic $[K^+]$ is tightly regulated in the region of 100 mM and E_m is about -120 mV, this would entail channel opening whenever $[K^+]_{ext}$ is in the mM range and closure at μM $[K^+]_{ext}$. As schematically shown in **Figure 2**, ORC gating properties ensure a progressive increase in channel activity when the K^+ gradient becomes outward, a property which *a priori* rules out a perceivable function of ORC in taking up K^+ from the external medium. By contrast, characteristics of IRC support the notion that IRC function as K^+ uptake system: IRC open whenever K^+ fluxes are inward, whereas the open probability diminishes and channel activation is effectively stopped whenever the K^+ flux becomes outwardly directed. [Nevertheless, the notion that IRC exclusively mediate K^+ influx and ORC only mediate K^+ efflux may be an over simplification (see below)].

Convincing evidence of channel involvement in K^+ uptake was obtained from complementation studies showing that growth of K^+ uptake deficient yeast cells was restored after heterologous expression of the *A. thaliana* K^+ channels *KAT1* and *AKT1*[1, 44]. Tissue expression studies showed predominant expression of *KAT1* in guard cells and vascular tissue whereas *AKT1* is mainly expressed in *A. thaliana* root cells[22]. The exact role of KAT1 and AKT1 *in vivo* is currently being studied, and there is now accumulating evidence that KAT1 plays a role in guard cell K^+ uptake and that AKT1 plays a role in low-affinity K^+ absorption in *A. thaliana* roots[22].

Native IRC have been characterized in the root plasma membranes of several species (e.g. 11, 27, 38, 43). In wheat (*Triticum aestivum*) root hair protoplasts, IRC mediated currents showed characteristics compatible with those of a low-affinity K^+ pathway[12]. In the majority of cells, E_m was more negative than E_{K+}, evoking large inward currents which remained stable over periods up to 10 minutes and were highly selective for K^+. An important argument to implicate this particular current in low-affinity K^+ transport stems from its high sensitivity to blockage by Al^{3+}: Aluminium inhibition was voltage independent and partly reversible, with a half maximal block at approximately 8 μM external free Al^{3+}. Although the mechanism of Al^{3+} toxicity is not known and its inhibitory effects are also exerted on transport of cations other than K^+, direct inhibition of channel activity provides one plausible explanation for the observed effects of Al^{3+} in intact roots where low-affinity K^+ uptake is similarly inhibited in the presence of Al^{3+}.

The plant K^+ status can have a profound effect on inducing or repressing the activity of root K^+ transporters[10, 13, 14] and such (compensatory) regulation will greatly enhance plant adaptability in coping with a varying supply of K^+. In *A. thaliana*[27, 29], K^+ starvation induced extra high and low-affinity K^+ transport capacity. High-affinity transport was augmented through an increased V_{max} and an otherwise unchanged K_m whereas the increased low-affinity uptake was mainly generated by a drop in K_m with the V_{max} unaffected[29]. The impact of the growth K^+ conditions on the various K^+ transport systems was exploited by setting growth conditions such that either $[K^+]_{ext}$ was sufficiently high (6 mM) to allow E_m driven uptake through channels or, alternatively, sufficiently low (100 μM) to prevent E_m mediated uptake. A change in K^+ growth conditions increased the activity of one particular species of IRC (with a unitary conductance of 6 pS

when $[K^+]_{ext}$ is 10 mM and $[K^+]_{cyt}$ is 100 mM). The enhanced IRC activity was paralleled by a raised Rb^+ uptake observed in intact roots. Although an increase in channel activity under conditions which effectively preclude channel mediated K^+ accumulation appears counter-intuitive, induction of extra 6 pS IRC activity and/or expression may be part of an overall response to low K^+ availability. Thus, boosted levels of inward K^+ channel activity can be beneficial to the plant when periods of stress are followed by abundant K^+ supply and channels, with a much larger K_m and V_{max} than carriers, ensure rapid replenishment of cellular K^+.

Additional data support the notion that specific channels are associated with low-affinity K^+ uptake. The selectivity sequences obtained for root IRC usually resemble $K^+ > Rb^+ > Na^+$ and are similar to those described for flux measurements in intact roots. Likewise, K^+ affinities determined for ion channels can be held alongside those recorded in intact roots: K^+ binding affinities in both wheat and *A. thaliana* for the respective whole-cell and single channel conductances are highly dependent on the $[K^+]_{ext}$. Kinetic analysis revealed that the K^+ dependent inward current in wheat root hairs saturates at a $[K^+]_{ext}$ of approximately 80 mM with an apparent K_m of 8.8 mM[12]. The half saturation constant of the 6 pS IRC in *A. thaliana* root cells is 19 mM with the single channel conductance saturating around 150 mM $[K^+]_{ext}$[27]. These values are in general accord with those observed for low-affinity K^+ uptake in intact roots (**Figure 1**). [Comparisons between data obtained in single cell studies and those from intact tissue measurements demand some caution: Kinetic parameters derived from root studies subsume effects of $[K^+]_{ext}$ on E_m (which alters the driving force on K^+ and also the activity of IRC), and therefore studies on whole roots yield phenomenological descriptions of kinetic parameters only.]

K^+ Channel Capacity

To test whether the channel mediated K^+ currents described above can explain the low-affinity K^+ fluxes which are actually measured in intact roots and plants, the properties of the single channel or whole-cell currents must be compared to those of the ionic fluxes measured in intact tissue. [For such comparisons a large number of assumptions is unavoidable (see 30), which complicates the interpretation of the data]. For the 6 pS *A. thaliana* IRC it was previously estimated that at a density of one channel per μm^{-2}, symmetrical 10 mM K^+, an open probability of 2 percent and E_m at -150 mV, the average IRC mediated current was well in excess of the measured Rb^+ influx in *A. thaliana* roots[29]. However in a subsequent more detailed study[30], it was shown that in most conditions the membrane voltage is only 15 to 20 mV more negative than E_{K+}. Therefore, both the driving force for K^+ and the (voltage dependent) channel open probability would be significantly smaller. Additionally, at the more physiological $[K^+]_{ext}$ of 1 mM, the IRC conductance will be reduced from 6 to 1 pS, and the proportion of root cells contributing to K^+ uptake may be as low as 20 percent. These more appropriate parameters reduced the IRC capacity to the extent that it became insufficient to explain K^+ uptake whenever $[K^+]_{ext}$ is in the lower mM range. Alternative mechanisms compensating for the lack of IRC capacity may include a (limited) contribution of ORC to mediate low-affinity K^+ uptake since small *inward* currents can be recorded through ORC in certain conditions[27,30].

Properties of Low-Affinity K^+ Uptake in Protoplasts and Intact Tissue

Although there are now compelling arguments in support of the notion that the low-affinity K^+ pathway comprises (specific) K^+ channels, several discrepancies remain between channel properties measured in protoplasts and low-affinity K^+ uptake characteristics observed in intact plants.

Firstly, the selectivity of the low-affinity pathway for K^+ over other monovalent cations has been reported to be fairly low in intact plants (see 7 for review). Low-affinity transport of K^+ in barley is competitively inhibited by the addition of Na^+, and in the absence of K^+, Na^+ uptake rates even exceeded those of K^+ alone[36]. Also, the use of Rb^+ as a K^+ analog inherently implies similar transport properties for these ions. Several reports, by comparing data obtained with $^{86}Rb^+$ and $^{42}K^+$[15], have shown that the low-affinity K^+ pathway does not discriminate between Rb^+ and K^+. In contrast, data obtained from patch clamp studies generally suggest higher degrees of ion selectivity in K^+ channels. In wheat root hair cells a K^+/Rb^+ selectivity of 5 was measured[12] whereas K^+/Na^+ selectivities of the dominant IRC in plant cells are generally high, ranging from around 6[27] to >100[12].

Secondly, the effects of Cs^+ on whole plant K^+ uptake are different from those in protoplasts. Considerable uptake of Cs^+ in intact tissue has been observed[6, 9] and it is assumed that Cs^+ enters the symplast through K^+ transporters[29, 45]. Yet Cs^+ is known to be a potent blocker of plant K^+ channels. In *A. thaliana* root cells IRC mediated K^+ currents decrease with a $K_{1/2}$ for Cs^+ of around 0.3 mM, and currents are inhibited to over 95 percent in the presence of 10 mM Cs^+[27]. In intact *A. thaliana*, a similar $K_{1/2}$ was established for Cs^+ inhibition of low-affinity Rb^+ uptake but around 20-30 percent of the Rb^+ uptake was found to be completely insensitive to Cs^+[29]. These results indicate that although the larger fraction of the observed low-affinity K^+ uptake in intact plants is probably carried by IRC, the remaining uptake proceeds through an unknown Cs^+ insensitive pathway.

In this respect it is also interesting to compare channel characteristics determined in heterologous expression systems with those found in native tissues. From its expression pattern established by GUS fusion, it was presumed that AKT1 plays a

role in K^+ nutrition[22]. The coinciding predominance of the 6 pS IRC in *A. thaliana* root cells[27] may suggest that the 6 pS IRC and AKT1 are manifestations of the same channel species. Several biophysical parameters indeed yield corresponding values for AKT1 and the 6 pS IRC. Nevertheless, the AKT1 K^+/Na^+ selectivity determined in yeast cells[3] is a factor 3 to 4 times higher than that for the 6 pS IRC in *A. thaliana* root cells.

High-Affinity K^+ Uptake

Different Energetic Mechanisms

Cloning of *AKT1* and *KAT1* led to a renewed discussion about the nature of high-affinity K^+ uptake in plants (e.g. 20). This emerged from the observation that yeast cells which were complemented with AKT1 were able to accumulate K^+ from micromolar external solutions[44], indicating that, at least in principle, ion channel mediated K^+ uptake was possible in both the low and high-affinity K^+ range. However, in plants the K^+ gradient becomes outwardly directed whenever $[K^+]_{ext}$ drops below 0.5 to 1 mM (see above), and in these conditions K^+ uptake can no longer be driven exclusively by E_m.

Mechanisms for 'active' high affinity K^+ transport had been described for other cell types and include: (i) Na^+:K^+ and H^+:K^+ exchange pumps in animal cells and some bacteria, which are fueled directly by ATP hydrolysis[23]; (ii) K^+ transport in symport with Na^+ in charophytic algae[48] where downhill movement of Na^+ provides the driving force; (iii) high-affinity K^+ accumulation coupled to down hill transport of H^+ as observed in *Neurospora crassa*[39]. It was frequently observed in plants that addition of K^+ to the external medium resulted in acidification (e.g. 46) with an apparent stoichiometry of $1K^+$ per H^+[2]. This observation may explain why most mechanisms proposed for higher plant K^+ transport incorporated this phenomenon (**Figure 3**). For example, ATP-fueled K^+-motive pumps (in combination with H^+-ATPases) or K^+/H^+ exchange pumps would be capable of mediating 'active' high-affinity K^+ uptake while simultaneously extruding H^+. However, the apparent interaction between K^+ and H^+ is likely to result from an electrical effect and shows a highly varying K^+:H^+ stoichiometry[34]. Furthermore, K^+ additions produce membrane depolarizations which must result from the electrogenic nature of K^+ uptake, and therefore argue against the operation of a simple electroneutral K^+/H^+ exchange mechanism.

To investigate the energetic principles of high-affinity K^+ accumulation, it is imperative to control the membrane voltage, the K^+ gradient, and the respective other gradients or compounds that may impact on the K^+ transporter. Flux and inhibitor studies in intact roots are therefore inadequate for a number of reasons. First, E_m is highly sensitive to the K^+ gradient[4, 5, 21, 25]. Any change in $[K^+]_{ext}$ alters E_m which in turn affects the very driving force for K^+ uptake. Second, inward current flow through the high-affinity K^+ uptake system is difficult to quantify since it will, to an unknown and probably variable extent, be recirculated as an outward current through the primary H^+ pump at the plasma membrane. Third, quantitative measurement of ionic currents in intact roots is not possible because of intercellular current spread through plasmodesmata and other pathways. Fourth, direct control of the cytoplasmic compartment is not possible in intact roots.

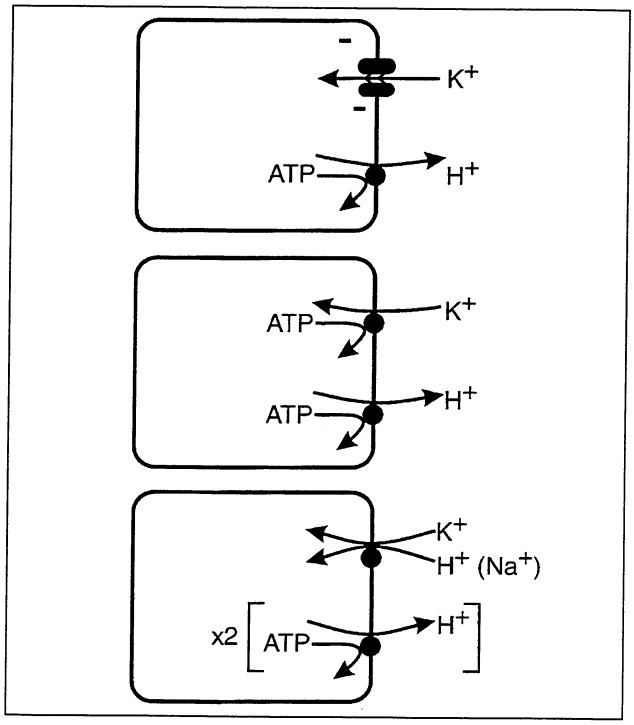

Figure 3. **Different mechanisms to explain the frequently observed exchange between K^+ and H^+. Top panel: uniport (channel) mediated K^+ uptake is driven by the negative membrane voltage and the influx of positive charge is compensated by the extrusion of a H^+. Middle panel: an ATP fueled pump is responsible for (high-affinity) K^+ accumulation, and the uptake of K^+ is compensated by primary pump activity. Lower panel, high-affinity K^+ uptake is driven by the H^+ (or Na^+) gradient, and the influx of two positive charges is counteracted by the extrusion of 2 H^+.**

High-Affinity K^+ Transport Coupled to H^+

An electrophysiological approach was applied to study high-affinity K^+ uptake in isolated *A. thaliana* root protoplasts[26]. Implementation of the whole-cell patch clamp configuration ensures complete control over the trans-membrane voltage and ionic gradients. Also, the cytoplasmic and external K^+ concentrations can be chosen such that the K^+ gradient is outward directed and, therefore, E_m driven K^+ uptake is prevented. Changing the cell bathing solution from K^+-free to μM K^+ elicited small, K^+ dependent inward currents. Increasing μM levels of K^+ in the bathing solution led to satu-

ration of inward currents at $[K^+]_{ext}$ values of 300 to 400 μM with an apparent K_m for K^+ of around 30 μM and an apparent V_{max} of 40 nA mm^{-2}. These figures agree well with data obtained for high-affinity K^+ transport in intact tissue. For instance in *A. thaliana* root cells, the apparent high-affinity K_m for Rb^+ uptake varies between 20 and 100 μM whereas saturation occurs around 0.5 mM $[K^+]_{ext}$ (**Figure 1**).

The mode of energisation of this high-affinity K^+ transport was examined by recording the absolute levels of K^+ dependent current and the reversal voltage (E_{rev}) of the K^+ dependent current as a function of different trans-membrane ionic gradients and cytoplasmic modulators. The E_{rev} voltage, at which current through the transporter reverses sign, can be used as a diagnostic to determine the ionic species that carry the current which is under investigation.

Modification of the trans-membrane Na^+ gradient, the Ca^{2+} gradient, or the levels of cytoplasmic ATP did not significantly affect the K^+ dependent current[26]. Therefore, the possibility that high-affinity K^+ transport is directly driven by an ATPase type pump or by cotransport with Na^+ or Ca^{2+} was discarded. In contrast, variation of the transmembrane H^+ gradient did have a distinct effect: A change in external (bath) medium pH from 4.5 to 5.5 produced a reduction in the average K^+ dependent inward current from 24 to 11 nA.mm^{-2}.

Although suggestive of coupling between K^+ and H^+ transport, these results could merely point to allosteric effects of the external pH on transport activity. However, in addition to the kinetic effects, pH changes also induced shifts in E_{rev} which provides strong evidence that K^+ and H^+ fluxes are interdependent. More formally, the E_{rev} of the overall transport reaction

$$K^+_{ext} + nH^+_{ext} \rightleftarrows K^+_{cyt} + nH^+_{cyt} \qquad \text{Eq. 2}$$

has to change in response to alterations in the K^+ *and* H^+ gradients and is defined for the equilibrium condition as:

$$E_{rev} = \frac{RT}{(n+1)F} \ln \frac{[H^+]^n_{ext}[K^+]_{ext}}{[H^+]^n_{cyt}[K^+]_{cyt}} \qquad \text{Eq. 3}$$

Here 'ext' and 'cyt' are, respectively, the external and cytosolic compartment, n is the transport coupling ratio, R and F are constants and T is the absolute temperature.

The E_{rev} measured at different K^+ and H^+ gradients closely conformed to eq. 3 assuming a coupling ratio of $n = 1$[26]. With a coupling ratio of 1 K^+ per H^+, high-affinity K^+ accumulation is theoretically capable of reaching values in excess of 10^6 times $[K^+]_{ext}$[26], a value that far exceeds those reported for higher plant cells (e.g., 6). From patch clamp experiments it can also be derived that a typical *A. thaliana* root cell with a diameter of 20 μM generates a K^+ dependent inward current in the region of 5 to 15 pA per cell or the equivalent of 20 to 60 μmol/gFW/h in intact root tissue (assuming the mechanism is constantly active, all cortical cells contribute, E_m = -100 mV, $[K^+]_{ext}$ =10 μM, and a 3 unit pH gradient[26]). This value is considerably higher than that observed in intact tissue where Rb^+ fluxes range from 0.5 to 2 μmol/gFW/h in intact *A. thaliana* roots at comparable $[K^+]_{ext}$ (**Figure 1**).

More recently, a high affinity K^+ transporter (HvHAK1) from barley was cloned[40]. Expression in yeast cells revealed a K_m for K^+ of around 30 μM, no or little discrimination between Rb^+ and K^+, and no effect on the uptake of Rb^+ into yeast cells by the addition of micromolar Na^+ (0 to 200 μM). In addition, cell exposure to mM levels of Na^+ decreased the Rb^+ uptake. The authors therefore concluded that HvHAK does not function as a K^+:Na^+ cotransport (see below) but most likely constitutes a K^+:H^+ symport similar to that observed in *A. thaliana*.

High-Affinity K^+ Transport Coupled to Na^+

As was described above for *A. thaliana* K^+ channels and a barley K^+:H^+ symport, yeast strains deficient in K^+ uptake provide a valuable vehicle to isolate plant genes that encode K^+ transporters. A cDNA library, prepared from root cells of wheat plants that were starved for K^+, was used to complement yeast cells deficient in high-affinity K^+ uptake[43]. Isolation of yeast strains exhibiting growth on low K^+ media allowed the isolation of the cDNA clone *HKT1* (for High-affinity K^+ Transporter). *HKT1* codes for a 534 residue protein that is predicted to span the membrane between 10 and 12 times. No ATP binding domains were detected, strongly arguing against the direct involvement of ATP in high-affinity K^+ uptake as was concluded for *A. thaliana*.

Initial characterization of *HKT1* by expression in oocytes showed a low degree of K^+ selectivity. Substitution of K^+ in the oocyte bathing medium with equimolar levels of Cs^+, Rb^+ and Na^+ induced currents which were roughly 80 percent, 60 percent and 40 percent respectively, of the K^+ induced current. Early characterization also led to the suggestion that *HKT1* mediated K^+ transport was dependent on the external H^+ concentration. A positive shift of 24 mV in E_{rev} was recorded after a 10-fold increase in the external $[H^+]$. A 10-fold increase in $[K^+]_{ext}$ generated a similar positive shift in E_{rev}. These values would indicate a coupling ratio n between 1 and 2 since, from rewriting equation 3 as

$$E_{rev}\,(mV) = \frac{58}{(n+1)} \log \frac{[H^+]^n_{ext}[K^+]_{ext}}{[H^+]^n_{cyt}[K^+]_{cyt}} \qquad \text{Eq. 4}$$

it follows that a 10-fold change in either K^+ or H^+ gradient produces a 29 mV shift in E_{rev} if n=1 and 19 mV if n=2.

However, subsequent characterisation in both yeast cells and oocytes revealed convincingly that HKT1 functions as a Na^+:K^+- rather than a H^+:K^+-

symport[41]. Radiolabeled Rb^+ uptake in yeast cells was strongly Na^+ stimulated ($K_{1/2}$ for Na^+ stimulation of around 175 μM), whereas in the same cells Na^+ uptake was stimulated by external K^+ ($K_{1/2}$ for K^+ stimulation of around 3 μM). Although some caution has to be taken in interpreting kinetic data obtained from heterologous expression systems (e.g., 16), these values clearly identified a high-affinity K^+-transporter. Furthermore, HKT1 mediated K^+-currents recorded in oocytes showed reversal voltages which varied in response to the K^+ and Na^+ gradients. Analysis of the reversal voltages according to equation 3 and 4 revealed a most likely coupling ratio of 2 K^+ per Na^+. The proposed stoichiometric ratio would sustain adequate K^+ uptake for levels of $[K^+]_{ext}$ as low as a few μM.

Na^+ coupled solute transport in plants is not unprecedented. Charophytic species exhibit high-affinity transport of K^+, urea and amino acids with a strict dependence on external Na^+[48]. Similarly, in several C4 species pyruvate transport over the chloroplast membrane similarly relies on the presence of cytoplasmic Na^+[37]. However, no data are yet available on the exact mechanism by which HKT1 functions *in planta*. In a recent report, the results from radiometric and electrophysiological assays conducted in various species including wheat and *A. thaliana* supported the conclusion that the presence of external Na^+ was not essential for either plant growth or high-affinity K^+ transport[31, 49]. Addition of Na^+ during the high-affinity K^+ transport assay was without effect or even inhibited K^+ absorption in wheat, barley, and *A. thaliana*. The latter characteristic was independent of growth conditions with respect to Na^+ status and pH. Even high pH assay conditions (pH 9), which would severely limit the driving force for H^+ coupled transport, failed to induce any Na^+ stimulation, and it was concluded that Na^+-coupled K^+ transport has no or limited physiological relevance in terrestrial species, whereas it may play a significant role in charophytes and a number of aquatic angiosperms[31]. It remains therefore a question whether HKT1 significantly contributes to high-affinity K^+ uptake *in vivo* or whether it constitutes an auxiliary mechanism responding to particular environmental conditions not yet tested.

Interactions between High and Low-Affinity K^+ Uptake

Adequate K^+ nutrition under conditions of widely varying $[K^+]_{ext}$ involves intricate regulation of all K^+ transporting moieties. Adaptive strategies employed by plants have been described in many reports (for review see 19 and 28) and include alterations in transport affinities and transport capacity as a response to altered levels in $[K^+]_{ext}$[24].

However, such adaptations occur with timescales of hours to days and probably involve *de novo* synthesis of protein. Yet, instantaneous K^+ uptake rates show a marked dependence on $[K^+]_{ext}$ (**Figure 1**) which requires the combined action of the low and high-affinity pathway. Intrinsic properties of K^+ channels and symporters may significantly contribute to the regulation of the overall K^+ flux, with the membrane voltage (E_m) being central in affecting both ion channel and symporter activity[30].

High-affinity K^+ uptake mediated by K^+:H^+ symport with a 1:1 coupling ratio carries two positive charges into the cell for every K^+ transported (**Figure 3**). It is therefore highly electrogenic and if the influx of positive charge is compensated by extrusion of 2 H^+ (**Figure 3**), it is also costly in energetic terms requiring the equivalent of two moles ATP per mole K^+. Additionally, with micromolar $[K^+]_{ext}$ the K^+ gradient is outwardly directed. Clearly these conditions require a minimised conductance through K^+ pathways other than the high-affinity pathway, since these would potentially function as a shunt and allow K^+ to leak out of the cell. In root cells, inward rectifying channels show gating characteristics which guarantee channel closure in these circumstances since the open probability becomes zero whenever E_m is more positive than E_{K+}[12, 27, 30, 42]. In contrast, ORC open probability may be considerable when E_m is moderately positive of E_{K+} and would thus form a potential K^+ leak pathway. However, the presence of micromolar levels of $[K^+]_{ext}$ and a cytoplasmic $[K^+]$ of around 100 mM constitutes a sizeable K^+ gradient which generates a large degree of open channel rectification. Thus, the very low level of $[K^+]_{ext}$ effectively restricts the K^+ outward conductance and so prevents K^+ leakage[30] (This is true only as long as E_m remains within 10 to 20 mV from E_{K+}, larger deviations from E_{K+} will create significant ORC open probability and will require alternative regulation schemes to ensure ORC closure.)

With millimolar levels of $[K^+]_{ext}$, channel mediated K^+ uptake becomes predominant. Uptake through this pathway carries a cost of 1 ATP or less per K^+ transported, operates with a high transfer rate, and is as such preferable over symport mediated uptake. This K^+:H^+ symport activity in *A. thaliana* root cells is strongly voltage-sensitive, with increasing transport rates at more negative E_m[30]. If symport K_m and V_{max} are affected by E_m[30] transport rates will drop considerably when the membrane depolarises. Millimolar levels of K^+ are well known to depolarise the membrane (e.g. 4, 5) and therefore limit symport mediated K^+ transport. For example, in *A. thaliana* root cells, a raise in $[K^+]_{ext}$ to around 5 mM depolarises the membrane by 80 mV[25] which in turn reduces K^+ uptake mediated by the high-affinity pathway to a few per cent of the total K^+ influx[30].

Additional environmental and intracellular factors may affect the absolute activity rates and the balance between high and low-affinity K^+ transport on a larger time scale. These will include cytoplasmic K^+[10, 13, 14, 47], phytotoxic cations that interfere with K^+ transporters such as Cs^+[29] and Na^+[36],

and a range of potential cytoplasmic modulators such as phosphorylation/dephosphorylation and redox potential.

Summary

Potassium as a macronutrient plays a crucial role in cell homeostasis, and the mechanisms responsible for its accumulation have been studied for many decades. The hypothesis, postulated over 30 years ago, that K^+ uptake in higher plant roots consists of two dominant mechanisms, is to a large extent confirmed by recent results.

The basis for low-affinity K^+ uptake which was originally termed 'facilitated diffusion' can now be observed on a single protein level in the form of ion channels which mediate 'passive' influx of K^+ down its electrochemical gradient. Specific inward rectifying channels in root cells form a permanent K^+ conductance as long as the electrochemical K^+ gradient is inwardly directed and are heavily implicated in low-affinity K^+ uptake. Voltage dependence and kinetic properties ensure channel closure whenever the K^+ gradient becomes outwardly directed.

High-affinity uptake shows all the classical features of carrier kinetics, and the notion of this process being directly energized is now corroborated by evidence of coupling to either the H^+ or the Na^+ gradient.

Synopsis of Future Research Imperatives

Application of powerful electrophysiological and molecular techniques has proved productive in enhancing our knowledge about the underlying principles of K^+ accumulation. The molecular properties of both high and low-affinity K^+ transporting mechanisms are rapidly being elucidated. Several ion channels have now been identified that play a major role in K^+ uptake. Evidence has been presented for H^+ coupled and Na^+ coupled symport to explain the basis of high-affinity K^+ uptake. Clearly, our view is not complete yet, and it cannot be excluded that roots contain additional K^+ transporters. Some of these mechanisms may have remained undetected due to their location in less accessible tissue whereas manifestation of other systems may only present itself in environmental conditions not yet tested. Furthermore, as was shown for the gene family encoding the H^+-ATPase, multiple isoforms may be distributed throughout the root tissues[40]. Indeed, the number of new K^+ channel homologs described in the literature increases steadily.

Technologies such as laser microsurgery and the use of gene fusion markers will assist in the isolation of specific cells which are amenable to electrophysiological assays. Such technology will facilitate surveying of spatial distributions within the root of particular K^+ pathways and also increases the opportunity to discover and characterise novel K^+ transport mechanisms. Additionally, the further cloning of genes involved in K^+ transport and its regulation enables isolation of transgenic plants where over-expression and introduction of antisense DNA will greatly enhance our knowledge on the physiological role of the respective mechanisms *in planta*.

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Chapter 5:
Roles of Higher Plant K^+ Channels

Frans J.M. Maathuis[1], Audrey M. Ichida[2], Dale Sanders[1], and Julian I. Schroeder[2]

[1]The Plant Laboratory, Department of Biology, University of York, PO Box 373, York YO1 5YW, U.K.
[2]Department of Biology & Center for Molecular Genetics, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0116, USA

Introduction

Living organisms maintain a cellular solute composition very different from that of the external environment. This implicitly requires transport of solutes across the cell membrane, and ion channels are integral membrane proteins which play indispensable roles in such transport. The past dozen years have witnessed radical advances in our understanding of ion channel function and regulation in higher plants. Nowhere are these advances more striking than with respect to K^+ channels, where the synergistic application of electrophysiological, cell biological, physiological, and molecular techniques has demonstrated an array of channel types suggested to play diverse, but defined, roles in plant physiology.

The major function of K^+ channels in animal cells is that of membrane voltage control and short term repolarization of the membrane. Although K^+ channels in plants share similar roles in regulation of the membrane voltage, early research on guard cells led to the model that plant K^+ channels in addition provide important pathways for long term physiological K^+ uptake and K^+ release. An extensive range of recent studies suggests diverse long term transport functions of plant K^+ channels including participation in osmotically driven movements, solute loading into the xylem, cation nutrition, and, by virtue of the presence of K^+ channels at endomembranes, intracellular solute redistribution and cytosolic volume control. Most plant K^+ channels remain activated for long periods of time which is critical for this proposed long term transport function of K^+ channels in plants. Because higher plant K^+ channels are proposed to play a role in regulating both the influx and the efflux of K^+ from cells, activity of these channels may impinge on aspects of turgor and water relations of all plant cells. In this update we focus on important principles of plant K^+ channel function and on the proposed physiological roles of specific plant K^+ channel types in the plasma membrane and tonoplast (**Figure 1A**) of different plant cells.

Ion Channels Defined

Ion channels catalyse transport through membranes at rates between 10^6 and 10^8 per second per channel protein. Transport is "passive". In other words, diffusion of ions through the channel is a function of both the membrane voltage and the concentration difference for the ion across the membrane and is not directly coupled to the input of other forms of free energy.

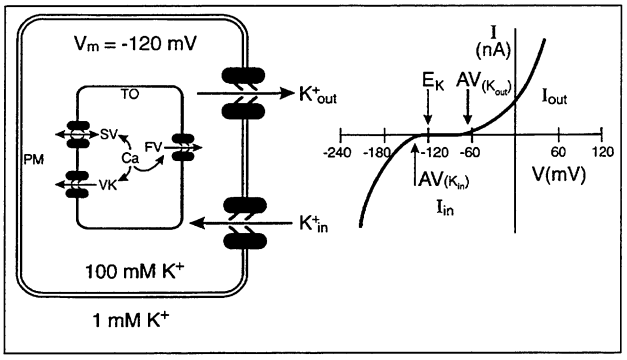

Figure 1. (A) A Schematic plant cell with the major K^+ conducting channels in the tonoplast (TO) and the plasma membrane (PM) reviewed here. (B) If the cell depolarizes to values positive of the K^+_{out} activation voltage (AV), K^+_{out} channels will progressively remain more in the open state and conduct an outward K^+ current (I_{out}). Membrane hyperpolarization to values more negative than the K^+_{in} activation voltage will lead to K^+_{in} channel activation conducting an inward K^+ current (I_{in}).

This simple picture of ions diffusing through a pore must be refined by consideration of two properties common to all ion channels. The first is selectivity. Selectivity implies the presence of binding sites for recognition of the ion during permeation, and channels are often named after the most permeant ion or after the ions of proposed physiological significance. However, it is important to recognize that selectivities are not absolute and that many channels will conduct a range of ions to some extent. This property is reflected in the so-called ionic selectivity sequence for the channel. The selectivity of a channel can have great physiological significance. Thus, some K^+ channels conduct Na^+ to a finite extent, and this could impact on the degree to which plants can withstand salinity (e.g. 47).

The selectivity of ion channels can be derived either by measuring the conductance of different ions through the channel or by determining the reversal voltage of the current through the channel (this is the membrane voltage where the net current is zero and reverses its sign). Selectivities derived from the two methods are not necessarily comparable, and in many physiological conditions they can yield different selectivity sequences[23].

The second universal property of ion channels is their ability to reside in "open" or "closed" conformational states which respectively either permit or not permit ion permeation. This conformational switching[23], can occur in response to ligands

or to a change in membrane voltage after which channels activate (open) or deactivate (close). Inactivation, i.e closing of open channels during continuous stimulation, is often observed in animal K^+ channels and represents a form of desensitization. The control of activation by membrane voltage or by ligands (e.g. Ca^{2+}) holds a key to understanding the roles of ion channels in cell biology. Two main types of K^+ channels have been identified, each with a characteristic voltage dependence (**Figure 1B**). One class of K^+ channels opens in more hyperpolarizing conditions (i.e. at rather negative membrane voltages) and was found capable of facilitating K^+ uptake based on its transport properties[53]. These channels are therefore known as inward-rectifying K^+ (K^+_{in}) channels (see 54). A second class of K^+ channel opens in depolarizing conditions and at these relatively positive voltages will carry an outward K^+ current. These are outward-rectifying K^+ (K^+_{out}) channels and have been described in both algae and higher plants (see 61). Detailed studies established that inward and outward rectifying K^+ currents in plant cells have different kinetics and pharmacological profiles, and it is generally accepted that the underlying channels are two different protein entities. Potassium channels comprise the dominant class of channel observed in a wide variety of plant cell types, including guard cells, aleurone cells, leaf cells, stem tissue, mesophyll cells, cortical and stelar cells, and root hairs, cortex, and stele[9, 15, 16, 15, 20, 37, 47, 53, 57, 65, 69, see 70 for rev. see 54]. Viewed in terms of both abundance and distribution, K^+ channels must clearly play some fundamental physiological roles in plant biology.

Structure of Plant K^+ Channels

The first identifications of K^+ channel cDNAs from plants were achieved by functional complementation of yeast cells which were defective in K^+ uptake. Two distinct *Arabidopsis thaliana* cDNAs, termed *AKT1* and *KAT1*, were independently cloned[2, 56], although both clones were structurally more homologous to nucleotide-gated than to voltage-gated channels. Still, they were shown to gate in a voltage dependent manner and show similarity in both amino acid sequence and protein structure to members of the so-called "Shaker" superfamily which comprises voltage-dependent, outward-rectifying K^+ channels from animal plasma membranes[26]. Similar to Shaker type channels, KAT1 and AKT1 hydropathy profiles predict six or seven membrane spanning regions (**Figure 2A**). Between the fifth and sixth membrane span, the P-domain is proposed to form the channel pore and contain binding sites for the permeating ions. The S4 transmembrane domain contains several basic residues spaced every third position. The positive charge on these residues comprises the voltage sensor which is required for the channel to gate open in response to voltage changes over the membrane[23, 26].

Functional channels probably consist of four α subunits of 65-100 kDa (**Figure 2B**) with the P-domain from each subunit lining the channel pore[23, 26]. In animal systems, β-subunits provide additional mechanisms to diversify K^+ channel properties by either modifying inactivation properties or by acting as chaperons[18]. In plant cells, similar regulatory functions could be fulfilled by hydrophilic subunits. An *Arabidopsis* homologue to animal K^+ channel β-subunits has been identified which may contribute to K^+ channel functioning[59]. Binding of β subunits to a guard cell membrane protein that is recognized by KAT1 antibodies supports this hypothesis[60].

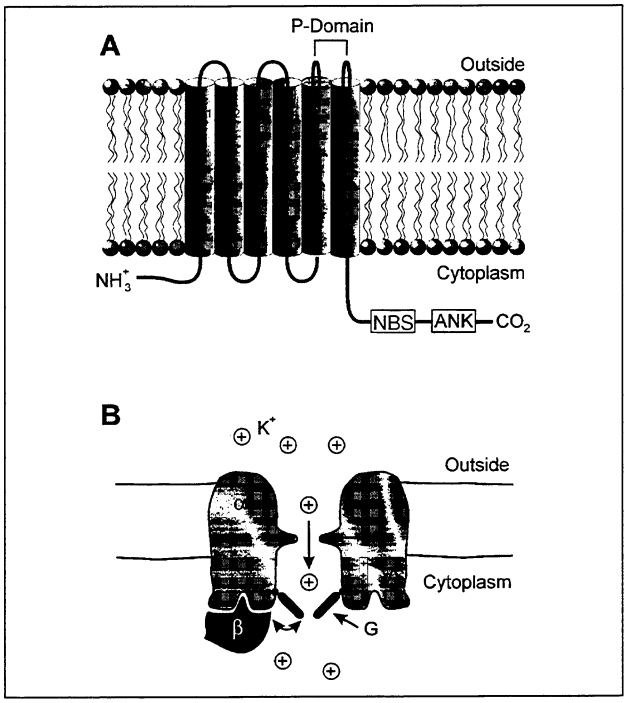

Figure 2. (A) Generalized structure of voltage-gated channels with six membrane spanning regions. A sensor in the S4 region reacts to the membrane voltage and contains several positively charged amino acids. The P-domain is thought to form part of the channel pore and is crucial in determining channel selectivity. At the COOH terminus, nucleotide binding sequences (NBS) and ankyrin like structures are present as AKT1 and AKT2 channels. (B) K^+ channels are thought to form tetramers of the main subunit (α) which contains the selectivity filter (SF), a voltage sensor (VS), and a gate (G). The voltage sensor and gate open or close the channel in response to alterations in the membrane voltage. Additional (β) subunits may have regulatory functions.

Despite the similarities to depolarization-activated K^+ (K^+_{out}) channels in animal cells, voltage clamp studies of KAT1 in *Xenopus* oocytes[48] and of AKT1 in yeast[6] demonstrated that both behave as inward-rectifying K^+ channels. The functional properties of KAT1 and AKT1 correspond to hallmark characteristics of K^+_{in} channels described in patch clamp studies of higher plant cells, including the time dependence, voltage dependence, K^+ dependence, cation selectivity, lack of inactivation, and Ba^{2+} and tetra-ethyl-ammonium (TEA^+) block[6, 48].

Homologous K^+_{in} channel cDNAs have been identified in *Arabidopsis thaliana* (*AKT2/AKT3*) and in potato (*KST1*)[10, 29, 44]. Molecular details of plant outward-rectifying K^+ channels are also beginning to emerge. An *Arabidopsis* K^+_{out} channel KCO1 was recently expressed in insect cells[13]. This channel showed a steep dependence on cytosolic free Ca^{2+} concentration and belongs to a new class of so called "two pore" channels[28].

Expression of different inward-rectifying K^+ channels is highly tissue-specific. Thus *KAT1* is predominantly expressed in guard cells[46] and *AKT1* is predominantly expressed in the epidermis, cortex and endodermis of the mature root, and also in hyadothodes[10, 31]. The potato *KAT1* homologue (KST1), is also expressed in guard cells and flowers, indicating conservation of tissue-specific expression in different species[44]. Expression of *AKT2* is predominantly in leaf tissue and at much higher mRNA levels in whole leaves than those determined for *KAT1*[10].

Cation Selectivity of Plant K^+ Channels

Cation selectivities for different K^+ channels vary widely, from highly selective for K^+ to a virtual absence of cation discrimination. Mutational analysis of the KAT1 K^+_{in} channel supports the model derived from animal systems in which the P-domain determines cation selectivity and channel block[3, 45, 63]. The ability to complement K^+ uptake deficient yeast mutants with plant K^+_{in} channels has added a new powerful genetic approach for identifying amino acids and structures involved in K^+ selectivity, which were not available in previous animal K^+ channel studies. An elegant genetic selection of mutants in yeast has pointed to the importance of the amino acid stretch glycine-tyrosine-glycine (GYG) for determining K^+ selectivity[3, 45]. Furthermore, mutations 5' of the GYG sequence (T256) were found to invert the specificity of KAT1 when comparing the K^+ conductance to the Rb^+ and NH_4^+ conductances. While the Rb^+ and NH_4^+ conductances of KAT1 expressed in oocytes are approximately 20 to 30 percent of the K^+ conductance[48], the mutations T256D and T256G evoked Rb^+ and NH_4^+ conductances that were 10 times larger than the K^+ conductance[63]. However, this large inversion in selectivity, as measured by conductance ratios, did not apply to the selectivity as measured by reversal voltage analysis[63], underlining the fact that the two different types of analysis are not comparable[23, 64].

Voltage-Dependent Gating

Both K^+_{in} and K^+_{out} channels are increasingly activated at respectively more negative and more positive membrane voltage. This voltage dependent gating results in current "rectification" (i.e. transport in mainly one direction). Inward rectification of animal K^+_{in} channels is achieved by selective blocking of outward currents through these channels with cytoplasmic Mg^{2+} or polyamines[23, 34]. Channel blockage by these compounds is voltage-dependent and becomes more pronounced at increasing membrane depolarization. However, studies of KAT1 expressed in *Xenopus* oocytes[24] and of *Vicia faba* guard cell K^+_{in} channels have shown that in plants inward rectification of K^+_{in} channels is not mediated by a similar mechanism but is likely to be controlled directly by the membrane voltage via the S4 voltage sensor.

The voltage sensor contains positively charged residues that react to changes in the membrane voltage. The number of charges in the channel protein that is displaced during channel opening (the gating charge) can be estimated with biophysical analyses and was determined to be 1.3 to 1.8 for KAT1[24, 64]. The crucial role of the S4 domain in sensing the membrane voltage also became apparent from studies where mutations in the S4 domain converted the animal Shaker K^+_{out} channel into a K^+_{in} channel[41]. Based on such observations a model was derived for the Shaker K^+_{out} channel in which mutations in the S4 domain can shift the voltage-dependence of parameters such as activation and inactivation to more negative values[41]. Ultimately, this shift would result in S4-dependent channel opening by hyperpolarization instead of depolarization and such a model could explain the high degree of similarity between KAT/AKT plant channels and Shaker-type channels, in spite of their opposite rectification characteristics .

However, some caution is appropriate in the interpretation of data obtained from heterologous expression systems. For example it becomes increasingly apparent that expression levels of plant K^+ channels can shift voltage-dependent parameters[10, 64], indicating that voltage sensing may also depend on the channel environment or local channel density. Furthermore, kinetic parameters may alter in different expression systems: The K_m for K^+ of KAT1 was considerably higher in oocytes than when expressed in yeast cells[30].

K^+ Channels Sense K^+

Potassium concentrations can affect the gating behavior of K^+ channels by modulating the threshold membrane voltage where channel opening starts, i.e. the activation voltage (**Figure 3B**). In guard cells, relatively little change in the activation voltage was observed when $[K^+]_{ext}$ changed in the range from 10 to 100 mM K^+. Yet, in the range from 0 to 10 mM K^+, the activation voltage does respond to alterations in $[K^+]_{ext}$[50]. Similarly, in *A. thaliana* root cells the K^+_{in} channel activation voltage always remained 20 to 30 mV negative of E_K[37]. Therefore, channel opening only occurs whenever the K^+ gradient is inward, suggesting that K^+_{in} channels function as a K^+ sensing valve allowing K^+ uptake only.

Additional unequivocal evidence that K^+_{in} channels actually do sense the external K^+ concentration originates from the observation that these channels no longer open when extracellular K^+ is

removed[20, 39, 50]. If K^+_{in} channels were not to sense external K^+, a very large outward K^+ current would emanate in K^+ free external solutions because of the large cytosolic K^+ concentration.

The ability of K^+_{in} channels to sense $[K^+]_{ext}$ is not absolutely K^+ specific. Thus, although KAT1 currents do vanish when $[K^+]_{ext}$ becomes zero[10, 48], after replacement of K^+ by Na^+ or Li^+, large outward K^+ currents can be generated by KAT1[10, 64]. Apparently, the K^+_{in} channel sensor also responds to cations of limited permeability such as Li^+ and Na^+, and further research on its physiological role would be of interest.

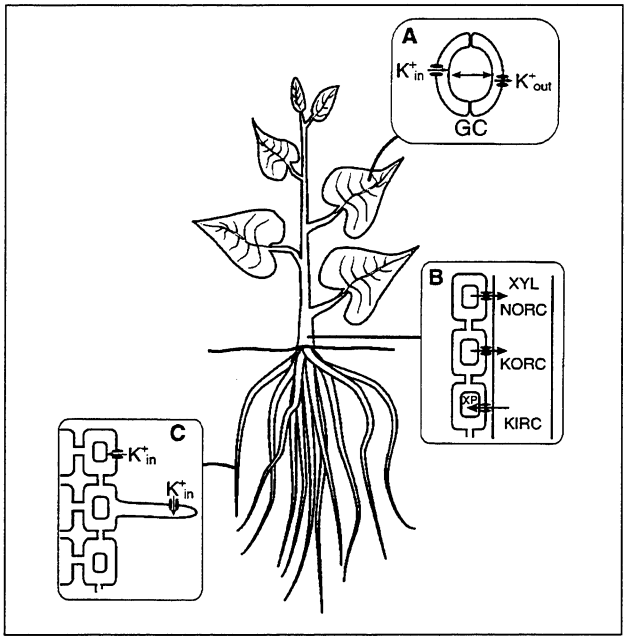

Figure 3. Potassium channels play essential roles in plants. (A) Regulation of the transpiration rate via adjustment of stomatal aperture crucially depends on transporting large amounts of K^+K^+ across the guard cell plasma membrane. Opening and closing of stomata involves K^+ transport through K^+_{in} and K^+_{out} channels, respectively. (B) Loading of the xylem vessel may depend on channels such as 'KORC' which release K^+K^+ into the vessel and may also play a role in the process of cation destination. In more mature parts of the xylem, resorption of specific ions is mediated by 'KIRC'-like channels and the non-selective channel NORC is believed to play a role in membrane voltage regulation. (C) Low-affinity uptake of the important nutrient K^+ takes place at the root soil solution interface and probably involves both epidermal and cortical root cells. Specific K^+_{in} channels have now been identified that form a pathway for such K^+ utake, provided the external K^+ concentration is sufficiently high to create an inward K^+ gradient.

Regulation of Membrane Voltage

In all biological membranes, the membrane voltage must be regulated within rather tight limits. This is the case for several reasons. First, the cytosol-negative membrane voltage provides the driving force for the electrophoretic transport of many solutes against their chemical gradients. This applies not just to important cationic nutrients such as NH_4^+, but also to neutral solutes such as sugars and amino acids whose transport is frequently coupled to that of H^+. Second, the membrane voltage is thought to play a key role in certain types of cellular signaling. For example, effectors can cause a membrane depolarization which in turn can trigger Ca^{2+} channel activation as part of a signaling pathway (for review see 67). Third, the membrane voltage is normally held in a range far positive of the reversal potential of the primary electrogenic H^+ pumping ATPase (which is around -450 mV). This enzyme generally functions continuously to remove excess metabolically-produced H^+ and part of the ATPase current may be short-circuited via ion channels to prevent the membrane voltage reaching too negative values.

The manner in which K^+ channels maintain membrane voltage can be assessed by calculation of the equilibrium voltage for K^+ from the Nernst equation

$$E_K = 59 \log [K^+]_{ext}/[K^+]_{cyt} \quad (1)$$

in which $[K^+]_{ext}$ and $[K^+]_{cyt}$ are the K^+ activities of the extracytosolic and cytosolic compartments, respectively, and E_K, the equilibrium voltage for K^+, is in mV. If K^+ channels comprise the dominant electrical conductance, the membrane voltage will approximate E_K. Typically in higher plants $[K^+]_{cyt}$ is of the order of 80 to 200 mM[36, 66]. In contrast to cytoplasmic K^+ concentrations which are tightly regulated, the K^+ content of soils varies highly, and this will affect E_K. For example, with values of $[K^+]$ in the soil solution and leaf apoplast ranging from around 500 μM to 10 mM, E_K would be -60 to -150 mV for a typical plant cell.

Resting levels of the plasma membrane voltage can become more negative than E_K, mainly depending on the extent to which activity of the H^+-ATPase hyperpolarizes the membrane. Inwardly rectifying K^+ channels exhibit a strongly increasing tendency to open as the pump drives the voltage more negative, and it is K^+ uptake through these channels which prevents the membrane voltage from becoming too negative.

Conversely, physiological stimuli induce rapid membrane depolarizations, for example, by anion and Ca^{2+} channel activation (see 67). Depolarizations positive of E_K will stimulate opening of K^+_{out} channels, and the resulting efflux of K^+ will tend to limit the extent of the depolarization. Subsequent additional activation of K^+_{out} channels gradually restores the membrane potential to a more negative value. These properties of K^+ channels in stabilizing membrane voltages are universal in eukaryotes and constitute the principal role of K^+ channels in animals where the repolarizing phase of action potentials is generated through opening of K^+ channels.

K^+ Channels and K^+ Nutrition

Potassium is the most abundant cation in most non-halophytic higher plant cells. Extensive studies on the kinetics of K^+ uptake into roots showed that K^+ uptake is mediated by at least two mechanisms, with high and low affinities for K^+ respectively (see 38). The ability of K^+_{in} channels to sense K^+ concentration is of major significance to the process of K^+ nutrition, and K^+_{in} channels have been described in different root cells including cortical, root hair, stelar, and xylem parenchyma cells[17, 20, 37, 47, 65, 69, 70].

In *A. thaliana* root cells, two types of K^+_{in} channel are observed[37]. Interestingly, activity of the predominant K^+_{in} channel (conductance 6 pS at physiological K^+ concentrations) was found to increase when K^+ levels in the growth medium were lowered to micromolar values.

Hallmarks of the *Arabidopsis* K^+_{in} channels are channel blockage by TEA, which has been shown specifically to inhibit low affinity unidirectional K^+ fluxes in corn roots, a selectivity ($K^+ \approx Rb^+ > Na^+ > Cs^+$) which resembles selectivities for unidirectional fluxes in whole tissue and enough K^+ conducting capacity to explain the influx observed in intact plants. In wheat root hairs and cortical cells, typical K^+_{in} channel currents were identified that show a similar K^+ selectivity[17, 20]. Furthermore, wheat root hair K^+_{in} channels were inhibited by Al^{3+} ($K_{0.5}$ = 8 μM)[20], which agrees with a previously established Al^{3+} sensitivity of K^+ uptake in intact plants. In both wheat and *A. thaliana* root cells, the K^+_{in} channel-mediated currents saturate as a function of $[K^+]_{ext}$ with an apparent Km in the range of 8 to 19 mM, values which correlate well with those determined for low-affinity K^+ absorption using tracers.

Two major overlapping functions have been proposed for these K^+_{in} channels: (1) They provide a pathway for low-affinity K^+ uptake, which is driven by the H^+ pump-established membrane voltage and (2) they contribute to membrane voltage control by (a) modulating the membrane conductance and (b) by sensing the soil-to-cell K^+ gradient, similar to a K^+ electrode (see above). The latter function in particular could influence nutrient uptake by other transporters[54].

Channel-mediated K^+ absorption can only proceed when the electrochemical gradient for K^+ is inward. However, generally the K^+ gradient becomes outward when $[K^+]_{ext}$ drops below 0.2 to 0.5 mM (see equation 1). In these conditions K^+ uptake must be active and is proposed to be mediated by symporters which act in parallel with channel-mediated K^+ transport. Therefore, any K^+ conductance forms a potential shunt pathway through which symport-accumulated K^+ can leak out of the cells, and it is imperative that K^+ channel conductance is minimized. As described above, K^+_{in} channels will be deactivated in these circumstances as a result of their K^+ sensing characteristics. However, activity of K^+_{out} channels is not necessarily zero at low $[K^+]_{ext}$ and overall conductance via this pathway is probably restricted by a dramatic reduction of the K^+_{out} open channel conductance[39].

Stomatal Movements

Changes in cell turgor are responsible for movement of cells and organs and are produced by transmembrane transport of large quantities of ionic and non ionic solutes. Examples include phototropic responses and the flytrap closure of insect-eating plants like *Diaonea muscilapa*, which is mediated by large and very rapid turgor changes in the trap lobe cells. A further example is the opening and closing of stomata which is a well characterized event that involves transport of K^+ and Cl^-. Patch clamp studies on *Vicia faba* guard cells demonstrated the abundance of K^+ selective single channels in the plasma membrane[52]. The early finding that these K^+ channels remained active during long recordings, together with their sufficient K^+ transport activity, led to the hypothesis that plant K^+ channels provide pathways for K^+ uptake and K^+ release, required in guard cells to osmotically drive opening and closing of stomata, respectively[52]. Two main types of K^+ channels were identified in guard cells of both *Vicia faba* and maize: the hyperpolarization-activated K^+_{in} channels proposed to mediate K^+ uptake and the depolarization-activated K^+_{out} channels proposed to mediate K^+ release [16, 53]. Biophysical studies on the cation selectivity, voltage and time dependencies, transport rates, lack of inactivation, and channel block by Al^{3+} supported the model that these channels provide important pathways for K^+ uptake and release during stomatal movements[53].

Studies on the modulation of guard cell K^+ channels by cytosolic factors have shown correlations between second messenger levels and regulation of stomatal movements. These cellular regulation mechanisms of guard cell K^+ channels and their physiological implications for stomatal movements are reviewed in more detail elsewhere[5, 67]. Examples are: First, ABA-induced Ca^{2+} elevation and the inhibition of stomatal opening by Ca^{2+}, which correlates with a downregulation of K^+_{in} channels by elevated Ca^{2+} concentrations[51]; second, enhancement of K^+_{out} channel activity by cytosolic alkalinization can be mediated by ABA-induced alkalinization[8, 25]; third, the activity of K^+_{in} channels is increased by extracellular acidification, which can provide an additional mechanism enhancing proton pump-induced stomatal opening[7].

In addition, several studies suggest that protein phosphatases play important roles in K^+ channel regulation. Calcineurin (PP2B) inhibitors disrupt the Ca^{2+}-dependent downregulation of guard cell K^+_{in} channels[35]. The K^+_{in} channel inhibition by the protein phosphatase inhibitor okadaic acid suggests that other protein phosphatases produce the opposite effect to that proposed for calcineurin[33, 62]. Furthermore, the characterization of the *ABI1* gene

as a protein phosphatase 2C homologue[32, 40] and effects of the *ABI1* gene on K^+ channels in transgenic tobacco[4] show a role for ABI1 in K^+ channel regulation.

Clearly, K^+ channel regulation is implicated as an essential component for the integrated response of stomatal movements. Nevertheless, there are two major reasons why modulation of K^+_{in} channel activity alone is not sufficient to determine stomatal opening.

First, both channel activation and sustained K^+ transport depend on a membrane voltage which is displaced from E_K since all K^+ transport will stop once the membrane voltage becomes equal to E_K (see equation 1). During stomatal opening the membrane will be hyperpolarized by the proton pump whereas K^+ efflux during stomatal closing requires depolarization which can be mediated by the activation of anion channels (see 5, 67).

Second, the activity of K^+_{in} channels, either before or after physiological stimulation, usually far exceeds the level of activity necessary to mediate the physiological K^+ influx observed during stomatal opening. For example, average absolute K^+_{in} channel currents in *Vicia faba* guard cells range from 100 to 500 pA for a membrane voltage of -150 mV. However, the average absolute K^+ fluxes during stomatal opening amount to only 8 pA in *Vicia faba*[53]. Indeed, a 90 percent block of K^+ channel current in *Vicia faba* guard cells only slightly slowed, but did not inhibit light-induced stomatal opening, providing support for models in which K^+_{in} channels are essential, but not rate-limiting during stomatal movements[27, 53]. This observation holds for K^+_{in} channels exclusively and not for K^+_{out} channels. In the case of K^+_{in} channels, proton pump mediated membrane hyperpolarization can compensate for partially blocked K^+_{in} channel currents by increasing the driving force for K^+ and increased channel activity. By contrast, the ability to depolarize the membrane (e.g. through anion channel activation) to enhance K^+_{out} channel activity is more restricted. One reason why K^+ channel activities are large under most conditions, may lie in other essential roles they play. Functions such as membrane voltage control and cellular homeostasis (see above), may all require some level of constitutive K^+ channel activity.

Another question relating to K^+ channel overcapacity concerns the possible existence of two types of inward-conducting K^+ channels in both laser-ablated *Commelina* guard cells[22] and in patch-clamped *Vicia faba* guard cells[71]. Both these channel types may be involved in stomatal control. This seeming surplus in K^+ channel capacity is also observed in other cells (e.g. in root cells) and its biological significance remains to be determined. Molecular physiological analyses of plant K^+_{in} channels will allow questions of possible redundancies in K^+ transport pathways to be addressed.

Leaf Movements

In nyctinastic (night closing) plants, rapid leaf movements occur in response to day-night changes. Night closing is under control of both light and internal clock stimuli, and is driven by the motor cells at the base of the leaf. Flexor cells shrink during leaf opening whereas opposite extensor cells swell. The reverse happens during leaf closure. Cell swelling and cell shrinkage involve respectively the uptake and release of large amounts of K^+, Cl^-, and other solutes.

A depolarization-activated K^+ channel in protoplasts derived from motor cells is capable of carrying the large K^+ currents that flow from shrinking cells, is sensitive to inhibition by TEA and quinine, and conducts several monovalent cations with a selectivity sequence $K^+ > Rb^+ > Na^+ » Cs^+ » Li^+$[43]. As explained above, to maintain a significant K^+ efflux, the membrane voltage must be more positive than E_K. Therefore, an anion channel activation may be a feasible mechanism to sustain K^+_{out} channel-mediated K^+ release in motor cells.

Motor cell swelling requires K^+ uptake. Stimulation of the H^+-ATPase will hyperpolarize the membrane and direct the K^+ gradient inward. Preliminary results indicate the presence of inward-rectifying channels in motor cells[41]. However, it has not yet been established to what extent these channels are K^+ selective. Opening of these channels would lead to K^+ uptake, but involvement of other (active) transport systems may be necessary to explain K^+ uptake in its entirety.

It remains unclear how environmental signals such as light are translated into a change in K^+ uptake. Modulation of the H^+-ATPase activity will play a crucial role in this. Interestingly, H^+-ATPases in opposite motor cells react to light in either a stimulatory or inhibitory manner, and this differential response would explain the necessary polarity in swelling and shrinking. Studies on how environmental signals such as light are translated into a change in K^+ transport have identified second messenger signaling mechanisms that coordinately regulate H^+ pumps and K^+ channels (see 12).

Loading/Unloading of the Xylem

Nutrients taken up by roots need to be transported to the xylem before they reach aerial parts of the plant. It was long thought that the stele did not actively participate in this process due to a lack of oxygen. However, it is now established that xylem parenchyma cells, which surround the xylem vessels, contain proton pumps, water channels, and ion channels, pointing to an active role of these cells in xylem loading/unloading[14].

In barley xylem parenchyma cells three types of cation channels have been identified[69]. Two channels called KORC (K^+ outward-rectifying conductance; equivalent to K^+_{out}) and NORC (non selective outward-rectifying conductance) become active

at membrane voltages more positive than -50 and +30 mV, respectively. NORC channels are non-selective among cations, therefore, similar to outward-rectifying channels described in endosperm cells[58]. Both KORC and NORC can co-reside in the same cell and their respective activation depends on the cytoplasmic Ca^{2+} level. In roots, the K_{out} channels are probably involved in release of K^+ into the xylem. Channels would operate whenever the cell becomes depolarized as a result of reduced pump activity and/or the opening of anion channels which will lead to the simultaneous release of anions into the xylem. The KORC channels also show a considerable conductance for Na^+, which could explain the occurrence of Na^+ in the xylem sap and shoots of NaCl grown barley. The low permeability for other (monovalent) cations indicates that it may also act as a 'filter', protecting the shoot from potentially harmful ions such as Cs^+ and Li^+. Comparable K^+_{out} channels, responsible for salt release into the xylem, were observed in the maize stele[47].

The NORC only weakly discriminates between cations and is active at high (> 1 μM) levels of cytoplasmic Ca^{2+} [14]. Its gating is not affected by external K^+ and its role in solute release may be limited. However, it could provide a function in maintaining the membrane voltage negative (see above).

The K^+ selective inward-rectifying K^+ channels in barley xylem parenchyma cells, named KIRC (equivalent to K^+_{in}), activate whenever the membrane voltage is more negative than -110 mV[69]. These channels potentially mediate resorption from the xylem into the xylem parenchyma rather than release of salts. Growing parts higher up in the plant form a sink for ions which have to be extracted from the xylem. Similarly, a role in resorption of harmful ions into specialized root cells is possible. A hyperpolarization of the membrane by the H^+-ATPase in these cell types would stimulate opening of KIRC and increase the driving force for passive cation resorption. Interestingly, KIRC showed a high permeability for Cs^+, an ion which normally blocks K^+ channels.

Vacuolar K^+ Channels

Non-woody plants derive their rigidity from cell turgor which is also the driving force for cell expansion. Accumulation of K^+ is a major contributor to the intracellular osmotic pressure, and with vacuolar K^+ activities normally comparable to those in the cytosol, up to 95 percent of cellular K^+ could reside in the vacuole. In most conditions vacuolar K^+ uptake requires active transport, whereas vacuolar K^+ release has been proposed to be mediated by K^+ channels.

Three major types of tonoplast K^+channels have been characterized. An ubiquitous type is the 'SV' (slow-activated vacuolar) channel which conducts K^+ into the vacuole[11, 21]. At normally observed tonoplast voltages and cytoplasmic Ca^{2+} concentrations, its open probability is extremely low. Consequently, a rise in cytoplasmic Ca^{2+}, which is frequently observed under stress conditions, could promote SV-channel activity. These channels were demonstrated to function as non-selective monovalent cation channels without a measurable anion permeability[11]. It was later established that apart from conducting monovalent cations, these channels allow Mg^{2+} permeation[1, 19] and have a significant Ca^{2+} permeability with tight Ca^{2+} binding properties[68]. SV channels were proposed to contribute to Ca^{2+}-induced Ca^{2+} release[68]. Furthermore, it was shown that SV channels function by binding multiple ions which are then transported through the pore in a single file[1, 19]. The multi-ion, single-file-pore mechanism of SV channels renders the proposed transport of anions across the membrane in the opposite direction[55], biophysically and thermodynamically unlikely.

A second type of K^+ channel has been found in *Vicia faba* guard cells, which is highly selective for K^+ and shows a cation selectivity sequence $K^+ >> Rb^+ > NH^+_4 >> Na^+ \approx Li^+ \approx Cs^+$ [68]. These vacuolar K^+ (VK) channels can be further distinguished from other tonoplast K^+ channels in that they are voltage-independent and activate at cytosolic Ca^{2+}-elevations in the physiological range[1, 68]. Such VK channels can conduct large K^+ fluxes from guard cell vacuoles to the cytosol and have been proposed to provide a pathway for vacuolar, Ca^{2+}-dependent K^+ release during stomatal closing. In this model, vacuolar H^+ ATPases would hyperpolarize the tonoplast to drive channel-mediated vacuolar K^+ release[68] and simultaneously contribute to the cytosolic alkalinization that occurs in response to ABA during stomatal closing[8, 25].

A third channel type is the fast vacuolar (FV) channel which is, like the SV channel, relatively nonselective amongst cations and possibly also for cations versus anions[21]. This channel opens at low cytoplasmic Ca^{2+} levels and is active at physiological tonoplast voltages. It mainly conducts cations from the vacuole to the cytoplasm and could therefore act to increase the osmolarity in that compartment[1, 21]. A recent study has demonstrated that FV channels could provide the pathway for vacuolar K^+ release during Ca^{2+}-independent stomatal closing[1] in guard cell vacuoles.

Conclusion

It is now abundantly clear that K^+ channels are involved in a range of physiological processes in higher plants. For the K^+_{in} channel these include:

(1) K^+ uptake into guard cells and into various plant cell types during cell expansion, movements and growth.

(2) K^+ nutrition and transport, by forming a low affinity uptake pathway in root cells.

(3) a possible role in xylem unloading by conducting cations from xylem to symplast.

(4) membrane voltage regulation, for example, by preventing excessive hyperpolarization.

Outward rectifying channels function in:

(1) membrane voltage regulation, by resetting the membrane potential after depolarizing stimuli or by preventing excessive depolarization.

(2) solute release, for example, to promote stomatal closure, tissue movements, and osmoregulation.

(3) xylem loading, by transporting cations from symplast to the xylem vessel.

Clearly these processes are under cellular control and are likely to be affected by the membrane voltage, levels of K^+ and Ca^{2+}, and phosphorylation. In spite of such proposed diversity in physiological functions, more specific identification of the roles of individual K^+ channels is now becoming possible by cDNA isolation, functional characterization in heterologous systems, and molecular physiological studies.

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Chapter 6:
Potassium and Sucrose in Guard Cell Osmoregulation

Lawrence D. Talbott[1], Sarah M. Assmann[2] and Eduardo Zeiger[1]

[1]Department of Biology, University of California, Los Angeles, Los Angeles, CA 90024
[2]Biology Department, Pennsylvania State University, University Park, PA 16802

Introduction

Stomatal movements result from the regulation of guard cell osmotic potential, which causes water to move between the guard cell and its external environment. The resulting changes in guard cell turgor are translated, via a specialized cell wall structure, into changes in the size of the stomatal pore. The fundamental role of osmotic potential was revealed in the mid-nineteenth century by von Mohl[55], who demonstrated that open stomata could be made to close by placing them in solutions of increasing osmotic strength. The accumulation or removal of solutes in the cytoplasmic and vacuolar compartments of the guard cell is an active process, and it is well documented that cyanide, DCMU, and other metabolic inhibitors abolish stomatal movement[83].

The nature of the solutes accumulated during stomatal movements remained a subject of speculation for most of this century. This was in large part due to the difficulty of conducting biochemical analyses on guard cells, which constitute a relatively small proportion of leaf cells and are dispersed throughout the epidermis. Prior to the late 1960s, guard cell osmoregulation was explained by the starch-sugar hypothesis, based on indirect evidence implicating carbohydrates as the most likely source of guard cell osmoticum. The independent discovery of K^+ fluxes in isolated guard cells[25, 26] focused attention on ion movements. There are now numerous studies documenting K^+ uptake in isolated guard cells and correlating K^+ accumulation with stomatal opening[3, 23, 37, 60, 61]. Uptake of K^+ was found to be driven by a proton motive force generated by a vanadate-sensitive proton ATPase[88, 105] and requires malate^{2-} accumulation[2] or Cl^- uptake[75, 76] to maintain electroneutrality. This body of research led to the formulation of the K^+-malate theory, which has virtually replaced the starch-sucrose hypothesis of guard cell osmoregulation in contemporary literature. Much progress has been made in characterizing ion channels of the plasma membrane and tonoplast involved in the movement of K^+ between the apoplast, cytoplasm, and vacuolar compartments. Current research efforts focus on regulatory mechanisms controlling these movements.

In addition, recent studies have discovered that sucrose plays a central role as a guard cell osmoticum and that carbohydrate metabolism comprises a significant alternative osmoregulatory system underlying stomatal movements. These findings pose a need to understand how carbohydrate metabolism is integrated into guard cell osmoregulation as a whole.

Potassium Use by Guard Cells

The initial 1943 discovery of guard cell K^+ uptake by Imamura[39] went largely unnoticed. Potassium uptake by guard cells and its correlation with stomatal opening[25, 26] was widely publicized in the late 1960s. Since that time, considerable effort has been focused on the role of K^+ accumulation in stomatal movements (for reviews see 59, 94). Potassium accumulation by *Vicia faba* guard cells has been assessed by histochemistry[23, 60], ^{86}Rb uptake[24, 25], flame photometry[3], and electron microprobe[37]. Based upon estimated osmotic potential requirements, several authors have calculated that the observed K^+ accumulation can account for a substantial portion of the solute requirements for opening. Malate^{2-} accumulation in *Vicia faba* guard cells has also been widely reported[2, 60, 62]. Malate^{2-} is presumed to be formed from the products of starch breakdown via the action of PEP carboxylase. Chloride may partially substitute for malate^{2-} when available[99].

Evidence for Multiple Osmoregulatory Pathways in Guard Cells

There are several lines of indirect evidence that K^+ and its counterions may not comprise the sole osmotica in guard cells. Studies in *Commelina* showed that a substantial portion of measured stomatal aperture could not be accounted for by guard cell K^+ content as measured by K^+-selective microelectrodes[51]. In addition it has been recognized that high concentrations of K^+ required for full opening would be toxic in the guard cell cytoplasm[59, 67].

Studies published over the past decade indicate that guard cells seem to possess at least three osmoregulatory pathways for solute accumulation (**Figure 1**). Pathway 1 involves the uptake of K^+ from the external medium and synthesis of the counterion malate^{2-} from carbon skeletons provided by the breakdown of starch. This pathway can be preferentially activated in isolated guard cells by low intensity blue light[91, 95] and is inhibited by KCN[83]. In isolated stomata, the operation of this pathway is restricted to the early phase of opening. At later times during blue light-stimulated opening, the K^+ and malate^{2-} content of the guard cells decreases and sucrose accumulates in conjunction with continued starch breakdown[65, 91, 95]. This

pattern of activity points to the operation of pathway 2, in which the carbon skeletons produced from starch breakdown are used for the synthesis of sucrose (**Figure 1**). Additional empirical support for this pathway comes from studies in which sucrose accumulation associated with starch breakdown was observed in *Commelina* stomata stimulated to open with fusicoccin[71]. Both pathways 1 and 2 would be dependent on metabolic energy, explaining the sensitivity of this opening to respiratory inhibitors, and appear to depend upon the activation of the blue light photoreceptor system in guard cells[106]. The action spectrum for malate^{2-} synthesis[50] has also been shown to match the action spectrum for blue light-stimulated opening[42].

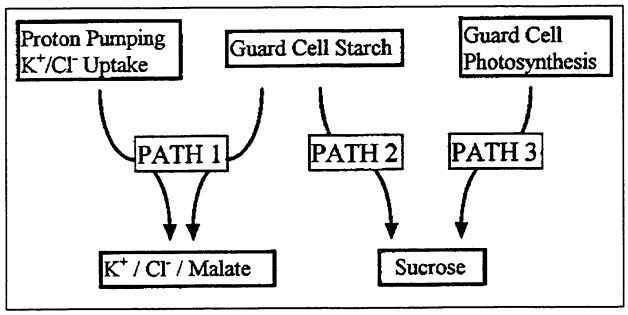

Figure 1. Osmoregulatory pathways modulating the content of K$^+$, sucrose and malate^{2-} in guard cells.

Pathway 3, seen with isolated stomata under red light illumination, involves sucrose accumulation without detectable K$^+$ uptake or starch breakdown[65, 91, 95]. In the absence of an external carbohydrate supply, guard cell photosynthesis is the most plausible source of this sucrose (**Figure 1**). Guard cells were previously thought to lack an operational Calvin cycle[59, 68]. However, Rubisco and the other Calvin cycle enzymes have now been detected in guard cells[89, 107]. Guard cell protoplasts irradiated with red light fix $^{14}CO_2$ into 3PGA[30], and guard cell chloroplasts show chlorophyll *a* fluorescence transients characteristic of photosynthetic carbon fixation[52]. Red light-induced opening of stomata is blocked by DCMU, an inhibitor of photosynthetic electron transport, and is insensitive to KCN. The three osmoregulatory pathways illustrated in Figure 1, although the best documented, do not represent the entire range of possible mechanisms. In the intact leaf, for instance, import of carbohydrate from mesophyll sources is a possible alternate source of osmotic sucrose.

Guard Cell Osmoregulation in the Intact Leaf

All of the investigations of stomatal osmoregulation discussed in the preceding section have used isolated guard cells, leaf disks, epidermal peels, or protoplasts. In contrast, the nature of stomatal osmoregulation in guard cells of intact leaves has received little attention. In a pioneering study, Pearson analyzed solutes in epidermal strips prepared directly from intact *Vicia faba* leaves at various times over the course of a daily cycle of opening[63]. Although interpretation is complicated by the existence of other cells in the epidermal preparation and by analysis using insensitive, colorometric techniques, this study found changes in both malate^{2-} and sucrose contents of stomata over the course of a light period.

A recent study examined guard cell osmoregulation during a daily cycle of stomatal movements in greater detail[92]. At various times during the course of a light cycle, abaxial epidermal peels of *Vicia faba* were prepared using sonication to isolate intact guard cells. Concurrent histochemical and HPLC measurements were used to measure the contributions of K$^+$, Cl$^-$, malate^{2-}, sucrose, and other monosaccharides to stomatal opening over the course of the light cycle. The use of electrochemical carbohydrate detection, which is both highly specific and very sensitive[41], permitted a much better quantification of individual carbohydrate species than was previously possible.

Guard cells accumulated K$^+$ rapidly during the initial phase of opening, in a pattern that generally matched the pattern of aperture increase (**Figure 2**). Guard cell K$^+$ levels subsequently declined, however, sometimes reaching initial baseline levels by midday. This decrease of K$^+$ content occurred at times of steady or even increasing apertures and was seen in plants grown under both greenhouse and growth chamber conditions. A second peak of K$^+$ accumulation, usually about half the amount of morning accumulation, was observed in the afternoon.

Afternoon K$^+$ uptake was variable in extent, ranging from 35-90 percent of morning levels. However, afternoon K$^+$ levels never appeared sufficient to account for the observed opening, as assessed by a comparison of morning and afternoon K$^+$/aperture ratios. A similar pattern of transient morning K$^+$ accumulation was seen in stomata of both greenhouse and growth chamber-grown onion cotyledons[5].

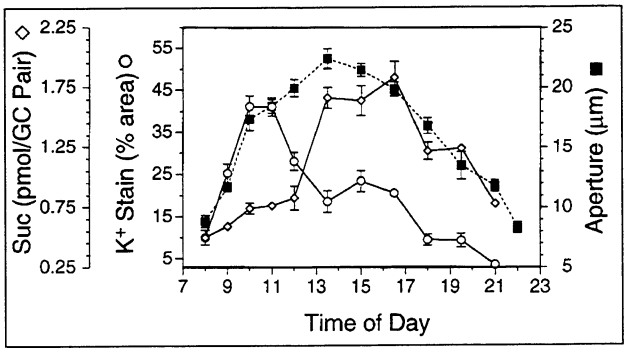

Figure 2. Aperture, K$^+$ and sucrose content of *Vicia faba* guard cells (GC) over a daily light cycle of stomatal movements. Plants were grown in a greenhouse environment. Data after (92).

Measurements of *Vicia faba* guard cell sucrose content revealed the opposite pattern of daily ac-

cumulation under both growth conditions[92]. Sucrose levels in the morning rose slowly and showed a variable amount of accumulation (**Figure 2**). Guard cells, however, showed a rapid accumulation of sucrose around the midpoint of the light cycle, and afternoon patterns of sucrose content and apertures corresponded closely. In particular, the pattern of stomatal closure closely matched the decrease in sucrose content of the guard cells whereas the pattern of K^+ loss was unrelated to closure. A midday closure of stomata was frequently observed in *Vicia faba* under greenhouse conditions. In this situation, maximal K^+ accumulation was correlated with the first peak of aperture while maximal sucrose accumulation was correlated with the second aperture peak (**Figure 2**).

Use of malate^{2-} and Cl^-, the two known counterions for K^+, differed depending on growth conditions. In growth chamber-grown *Vicia faba*, malate^{2-} content of guard cells peaked during the morning and declined at midday, in a pattern closely matching that of K^+ accumulation[92]. Minimal changes in Cl^- content of guard cells were seen throughout the course of the light cycle. Under greenhouse conditions, however, malate^{2-} accumulation was detected only during a predawn phase of opening. Substantial Cl^- accumulation was detected, with maximal accumulation occurring during the morning and correlated with maximal K^+ staining. The patterns of guard cell K^+ and Cl^- accumulation were always closely correlated throughout the light cycle in these plants, despite day to day variations in the ratio of morning and afternoon K^+ content.

Potassium Osmoregulation

This section will review the current understanding of the osmoregulatory mechanisms controlling the accumulation and efflux of K^+ and its counterions.

K^+ Fluxes at the Plasma Membrane

The mechanism of guard cell K^+ uptake through ion-selective channels has been the subject of recent extensive investigation. Electrophysiological assays of K^+ currents have predominantly been conducted using *Vicia faba* guard cell protoplasts, although the few extant studies on guard cells of maize, *Commelina communis,* and tobacco[8, 10, 22] show that K^+ channels of these species exhibit features qualitatively similar to those of *Vicia faba* (**Figure 3**). In all cases, two major types of K^+ selective channels have been observed in the guard cell plasma membrane: slowly-activating inwardly-rectifying K^+ channels that open to allow K^+ uptake and slowly-activating outwardly-rectifying K^+ channels that mediate K^+ efflux (see chapters 3, 4 and 5 in this volume for a definition of rectification). Potassium-selective channels that are stretch-activated have also been detected[18], but their relative contribution to the whole-cell K^+ flux remains unquantified.

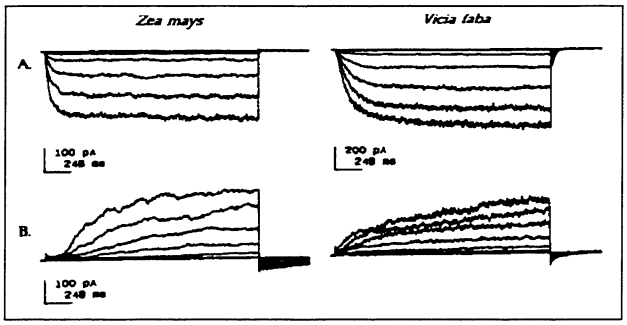

Figure 3. **Inward (A) and outward (B) K^+ currents in guard cell protoplasts of *Zea mays* and *Vicia faba*. Currents were elicited by sequentially stepping the membrane potential between -60 mV and -180 mV in -20 mV decrements (A) or from -40 mV to +80 mV in +20 mV increments (B). Note that the currents have a similar general profile in the two species, but differ in their magnitudes and rates of activation over time. Data from (22).**

The stretch-activated K^+ channels of guard cells have yet to be identified at the molecular level, but an inward K^+ channel gene, KAT1, has been cloned from *Arabidopsis thaliana* by functional complementation of a yeast mutant deficient in K^+ uptake[6]. Electrophysiological analysis of KAT1 expressed in oocytes has confirmed the inwardly-rectifying nature of the KAT1 channel[72], while use of the β-glucuronidase reporter gene fused to the KAT1 promoter has indicated that KAT1 is expressed primarily in guard cells[57]. A homologous gene, KST1, has been cloned from potato[56]. The availability of such clones has allowed the generation of mutant channels by site-directed mutagenesis or PCR, and these channels can then be analyzed to identify the structural aspects of the channel protein that confer K^+ selectivity[13, 80, 98] and rectification[13, 17, 36]. In animal systems, it has recently been discovered that while expression of a single type of K^+ channel subunit, the α subunit (analogous to KAT1), is sufficient to obtain a functional K^+ channel in *in vitro* expression systems, *in vivo*, an additional β subunit present in some systems interacts with the α subunit and influences the biophysical properties of the channel[1]. There is evidence from gene sequence homology[96] and *in vitro* studies of protein/protein interactions[97] that a β subunit is expressed in some tissues of Arabidopsis. Functional studies are needed to determine the role, if any, of the plant β subunit in the regulation of the KAT1 channel.

Ion flux through channels is passive, i.e., energetically downhill. Given estimates of intracellular K^+ concentrations in guard cells that are one-hundred to several-hundred fold greater than apoplastic K^+ levels, the prevailing K^+ concentration gradient would oppose passive uptake of this ion. Since K^+ uptake incontrovertibly occurs in some situations, channel-mediated uptake must therefore be driven by an electrical driving force. Specifically, both red and blue light have been shown to activate a H^+ ATPase at the guard cell

membrane[11, 45, 86, 87]. Hydrogen extrusion hyperpolarizes the membrane potential, i.e., makes it more negative, and this drives K^+ uptake. Reported magnitudes of red light-stimulated H^+ pump current are somewhat smaller than those for blue light, perhaps correlating with observations of reduced levels of K^+ accumulation under red vs. blue light.

Closing of plasma membrane anion channels may be a necessary adjunct to H^+ pump activation if K^+ driven stomatal opening is to occur[79, 85, 101]. Given that the electrochemical gradient across the guard cell membrane favors passive Cl^- and malate^{2-} efflux, loss of these negative ions through open anion channels would oppose the hyperpolarization necessary to energize K^+ uptake. It should be noted that a requirement for anion channel closure during stomatal opening does not preclude anion (e.g. Cl^-) uptake, which is known to occur under some conditions. Anions may be taken up in a carrier-mediated process[104], and this process need not affect the membrane potential if it is coupled to stochiometrically equivalent H^+ backflux or OH^- efflux.

Conversely, when the membrane potential depolarizes (becomes less negative) this will contribute to establishment of an electrochemical gradient that drives K^+ efflux during stomatal closure. Mechanisms of membrane depolarization in guard cells include H^+ ATPase inhibition, opening of Ca^{2+}-permeable channels and consequent Ca^{2+} influx, and opening of anion channels, resulting in malate^{2-} and Cl^- efflux. A dominant role of anion channel opening in membrane depolarization has been proposed[101]. It is certainly logical to postulate an importance of anion channels in this process, given that anion efflux is necessary to create the reduced Cl^- levels seen in closed stomata and also appears to contribute to the diminution of malate^{2-} levels as stomatal closure occurs[99].

Given the central role of K^+ in certain phases of stomatal response, it is not surprising that both inward and outward K^+ channels are regulated by a variety of signals. The overriding regulation is by voltage (membrane potential) itself[77]. The probability that a given inward K^+ channel will be open increases as the membrane is hyperpolarized, e.g. by blue light-stimulation of the H^+ ATPase, and this leads to proportionately greater K^+ influx as the more negative potentials are reached. In the case of the KAT1 polypeptide, such voltage-regulation has been shown to result from an intrinsic, voltage-sensing property of the channel itself[33, 81]. Conversely, the probability that a given outward K^+ channel will be open at any particular point in time increases as the membrane is depolarized, and this leads to proportionately greater K^+ efflux as membrane potentials become less negative. External pH is another parameter that affects K^+ channel behavior. A decrease in external pH favors activation of inward K^+ channels[15, 38], and this fits nicely with the premise that H^+ extrusion during stomatal opening causes a localized decrease in apoplastic pH in the region of the guard cell membrane.

Despite evidence discussed above for light regulation of K^+ uptake, a direct effect of light on guard cell K^+ channels has yet to be documented. To date, the major signal that has been studied as a regulator of both inward and outward K^+ channels is the phytohormone, abscisic acid (ABA). ABA application results in decreased inward K^+ currents and enhanced outward K^+ currents[14, 16, 47, 84], consistent with ABA's known effects as an inhibitor of stomatal opening and promoter of stomatal closure. It should be noted that the effects of ABA on guard cell ion transport are complex and not limited to action on K^+ channels. ABA inhibits proton pumping[29]. There is also indirect evidence suggesting that this plant hormone enhances opening of anion channels in the guard cell plasma membrane[74, 79, 85].

The ABA-inactivation of inward K^+ channels appears to be a multistep signal-transduction pathway which may have several branches. An ABA exposure sometimes, but not always, results in elevation of cytosolic Ca^{2+}, and such elevation has been reported to inhibit inward K^+ channels (see chapter 9 for review). These observations suggest that there could be both Ca^{2+}-dependent and Ca^{2+}-independent pathways for K^+ channel inhibition by ABA. The Ca^{2+}-dependent pathway appears to involve Ca^{2+}-release by the lipid-derived second messenger inositol 1,4,5 trisphosphate (IP_3). The IP_3 levels are transiently elevated in guard cell protoplasts treated with ABA[46], and introduction of exogenous IP_3 to the guard cell cytosol results in elevated Ca^{2+} levels[27] and inhibition of inward K^+ channels. In animal systems, IP_3 production is mediated by the enzyme phospholipase C. Some isoforms of this enzyme are G-protein activated, whereas others are regulated by G-protein independent mechanisms. In guard cells of *Vicia faba*, 3 out of 4 studies with G-protein regulators have shown that G-protein activators such as GTP γS inhibit the activity of inward K^+ channels[7, 21, 103] while the 4th study showed variable effects[44]. These results are consistent with a scenario whereby guard cell phospholipase C is activated by ABA via a G-protein. This, in turn, results in IP_3 production, Ca^{2+} elevation, and inward K^+ channel inhibition. However, it should be cautioned that a direct link between ABA exposure and G-protein activation has not been established. Indeed, the recent observation that cytosolic ABA can inhibit inward K^+ channels[84] is at odds with the dogma established from animal systems whereby G-proteins mediate signals emanating from plasma membrane receptors that bind external ligands. Finally, independent of the above mechanism of Ca^{2+} release via intracellular stores, there is evidence that ABA also promotes Ca^{2+} influx from the apoplast through Ca^{2+}-permeable channels in the guard cell plasma membrane (see 101 for review).

The power of molecular genetics is being brought

to bear on ABA regulatory pathways in guard cells. The ABA insensitive mutant of Arabidopsis, *abi1-1*, (reviewed in 28) exhibits a wilty phenotype, as would be predicted if stomatal closure were impaired. The ABI1 polypeptide is a phosphatase (see 28 for review), and protein de-phosphorylation is a common mechanism of signal transduction in general and ion channel regulation in particular[48]. Transformation of the dominant mutant allele *abi1-1* into tobacco results in guard cells with two to six-fold reduction in the magnitude of K^+ efflux currents and insensitivity of both inward and outward K^+ currents to ABA[8]. The insensitivity of both channel types to ABA implies that the signal transduction chains linking ABA to inward and outward channel activity have in common the *abi1-1* gene product. However, the pathways do not appear to be identical, as outward K^+ channel activity is not enhanced by increased cytosolic Ca^{2+} levels. Instead, H^+ concentration may comprise the ionic second messenger for ABA effects on the outward K^+ channel. An increase in cytosolic pH occurs upon ABA application [40], and artificial elevation of cytosolic pH results in enhanced K^+ efflux currents[16]. In isolated membrane patches, alkalinization increases the number of outward K^+ channels available for activation[54], which can account for the enhanced K^+ efflux. The fact that a pH effect on channel activity can be observed in the isolated membrane patch indicates that the pH effect on the channels is "membrane-delimited" and may even result from a direct effect of pH on the conformation of the channel protein, although this has yet to be tested.

Potassium Fluxes at the Tonoplast

It is presumed that the majority of the K^+ taken up during stomatal opening is stored in the guard cell vacuole[59, 67]. Given that: 1) the vacuolar lumen is positive with respect to the cytosol by approximately 20 to 50 mV due to the action of tonoplast ATP and PP_i-dependent H^+ pumps[70, 101] and 2) that vacuolar K^+ concentrations in open stomata are thought to exceed cytosolic K^+ concentrations by 5 to 10 fold[37, 51, 64], vacuolar K^+ accumulation must occur by active or energy-coupled transport mechanisms. The actual K^+ transport proteins, however, have yet to be identified for guard cells.

Conversely, K^+ loss from the vacuole as occurs during stomatal closure can be mediated by passive transport mechanisms such as ion channels. Three major K^+ permeable channels have been identified in the guard cell tonoplast to date. These are the fast vacuolar (FV), vacuolar K^+ (VK), and slow vacuolar (SV) channels, with "fast" indicating instantaneous activation and "slow" indicating time-dependent activation. Which of these three channel types dominates as the K^+ efflux pathway from the vacuole appears to depend on cytosolic conditions[4]. For example, activity of the FV channels is inhibited by Ca^{2+} but is promoted by cytoplasmic alkalinization[4, 49]. Conversely, the VK channels are inhibited by alkaline pH, but these channels are activated when cytosolic Ca^{2+} is elevated beyond 100 nM[4, 100]. At still higher cytosolic Ca^{2+} levels, beyond 600 nM, SV channels are activated[4, 82]. The SV channels mediate both Ca^{2+} and K^+ efflux, and may, therefore, create a positive feedback situation involving Ca^{2+} -stimulated Ca^{2+} (and K^+) release[100]. The SV channels were originally thought to be Cl^- permeable as well[31], but more recent experiments indicate that this may not be the case[4, 100].

Given the channel modulation described above, we may postulate that the FV channels are responsible for K^+ release in response to Ca^{2+} -independent, pH-dependent signaling, such as appears to occur along one branch of the ABA signal transduction chain. Indeed, in terms of their pH regulation, the FV channels appear to function analogously to the plasma membrane outward K^+ channel. The VK and SV channels are Ca^{2+}-stimulated, and thus presumably play a role in K^+ release following signals that elevate cytosolic Ca^{2+}. While ABA has received the major attention in this regard, ABA is not the only signal of this type; elevated CO_2 and oxidative stress can also increase cytosolic Ca^{2+} concentrations in guard cells[53, 102].

Malate^{2-} and Chloride Fluxes

It has been established that isolated guard cells can use differing proportions of Cl^- and malate^{2-} as counterions to balance K^+ uptake. In isolated stomata from *Vicia faba*, Cl^- was used to balance from 0 percent to 45 percent of accumulated K^+, depending on the external concentration of Cl^- provided[69]. It was proposed that Cl^- supply exerted a major effect on the species of counterion used[99]. The factors governing the use of these counterions in intact leaves is likely to be more complex. In the greenhouse and growth chamber conditions used in the HPLC study of stomata from intact plants[92], guard cells of greenhouse plants accumulated Cl^- in preference to malate^{2-}. Plants were well fertilized under both growth conditions, making it unlikely that Cl^- availability was a factor in the observed differences. Because apertures are regulated by different environmental stimuli in these two growth conditions[93], it is possible that different stimuli selectively activate the malate^{2-} production and Cl^- uptake pathways. Alternately, a specific environmental factor, such as light spectral quality, may control the availability of these pathways. The growth chamber environment is enriched in blue light[50], and blue light is known to preferentially stimulate malate^{2-} production[58]. However, the levels of malate^{2-} found in growth chamber-grown guard cells were substantially less than those found previously in isolated stomata of epidermal peels[91, 92]. Maximum malate^{2-} accumulation in guard cells from growth chamber-grown leaves was approximately 6.9 fmol/μm of opening, as opposed to the 43 fmol/μm found in guard cells from epidermal peels. Stomata of greenhouse-grown leaves, which

most closely approximate those from a natural environment, do not show any substantial malate^{2-} accumulation over the light cycle. These results indicate that extant information on the involvement of malate^{2-} in the balance of K^+ charge might be specific to guard cells in detached epidermal peels and that Cl^- may have a greater role in balancing K^+ than is currently thought.

During stomatal closure, levels of guard cell anions decrease. Malate^{2-} levels may decrease through catabolism or reconversion to starch[10]. In addition, both malate^{2-} and Cl^- levels may drop due to efflux through plasma-membrane anion channels. Anion channels with two different types of voltage regulation have been characterized in guard cells and have been named R-type (rapid) and S-type (slow) based on their kinetics of activation[43, 78]. Some data suggest that there is actually just one type of anion channel, which can be interconverted between the two different modes of voltage regulation[19]. Both R-type and S-type channels are permeable to both malate^{2-} and Cl^- [32, 73]. Channels functioning in the S-type mode may play the predominant role in anion efflux during stomatal closure simply because they remain activated for relatively long periods of time and thus could create the sustained depolarization necessary to drive the anion and K^+ efflux that accompanies stomatal closure.

Potassium-Sucrose Interactions in Guard Cell Osmoregulation

The existence of two phases in guard cell osmoregulation, an initial K^+ phase and a subsequent sucrose phase (**Figure 2**), implies the existence of a functional distinction between use of the two solutes. The close association between opening and K^+ uptake could suggest that K^+ accumulation is used primarily for rapid opening, whereas sucrose is used more in a turgor maintenance role. Although ionic movements are fairly rapid, rates of carbohydrate producing processes, such as starch breakdown in mesophyll[90], do not appear to be sufficient to support observed carbohydrate accumulation rates in guard cells. The possible relation between rapid opening and the use of K^+ was tested in experiments in which open stomata were closed and then rapidly reopened by manipulation of ambient CO_2. Morning reopening was accompanied by rapid K^+ accumulation, whereas reopening in the afternoon, sucrose-dominated phase was accompanied by rapid sucrose accumulation with very little participation of K^+[92]. There were no observed differences in the kinetics or extent of opening in the two phases. These experiments indicate that any functional distinction in the use of the two solutes probably does not include a limitation on the rate of accumulation.

Since osmoregulation consistently shifted from a K^+ to a sucrose dominated mode around midday in the daily timecourse experiments (**Figure 2**), it is possible that some circadian rhythm-driven mechanism may determine which osmoregulatory pathway is activated. This hypothesis was tested by using elevated CO_2 to suppress stomatal opening throughout the morning hours. Return of CO_2 to normal levels in the afternoon generated an experimental treatment in which the first substantial stomatal opening occurs in the sucrose-dominated phase. Stomata undergoing this treatment showed an initial opening dominated by K^+ uptake, thus arguing against a strictly circadian control of osmoregulation. It should be noted that suppression of opening by high CO_2 does not result in complete stomatal closure or a substantial reduction in mesophyll photosynthetic rate, thus eliminating the possibility that sucrose supply was limiting.

Taken together, the daily cycles and CO_2 experiments suggest an osmoregulatory sequence that requires K^+ to be used first. Potassium accumulation to some critical level may trigger a shift toward sucrose-based osmoregulation, initiating K^+ efflux and a metabolic state in which the guard cell has a reduced capacity to employ K^+. This regulatory sequence would be reset by a period of closure in the dark. Functionally, osmoregulatory pathways producing sucrose could be more tightly coupled to photosynthetic rate in the mesophyll. This would represent a mechanism whereby stomatal apertures could be more finely tuned to the rate of carbon fixation during midday and the early afternoon when a fine adjustment between leaf photosynthesis and stomatal conductance would result in more efficient water management for the plant. The K^+ pathway, on the other hand, would be active during morning opening, which occurs regularly as the sun comes up, but under conditions where light to drive photosynthesis would be limited.

Implications for Environmental Sensing

The existence of distinct phases of guard cell osmoregulation has important implications for the mechanisms whereby guard cells sense environmental cues. Much of current thinking has centered around the concept that specific environmental signals would be transduced into the activation of a specific osmoregulatory pathway. Thus a connection has been sought, for instance, between CO_2 concentration and the function of the malate^{2-} producing enzyme, PEP carboxylase[67]. However, the results of the CO_2 pulse experiments described above, which show that a single environmental signal has the capability to activate more than one pathway, indicate that environmental signals are not solute specific. Thus, a system of direct pathway activation would require that each osmoregulatory pathway have its own set of environmental sensors.

The ability of a single environmental signal to control multiple osmotic pathways raises the possibility that environmental signals are transduced

into apertures through a solute-independent parameter such as guard cell turgor. Under this scheme, single environmental signals would modify the activity of a turgor sensitive system, such as stretch activated channels sensing plasma membrane tension, the activity of which would be a signal for modifying aperture. Prevailing metabolic conditions would then determine which osmoregulatory pathway(s) are activated to generate the required solutes. This would result in an extremely flexible system in which solute generation could be adapted to factors such as light quality, availability of ions, or cellular starch content.

Future Research Directions

Progress in the field of stomatal osmoregulation will require both research into the mechanisms of K^+ and sucrose accumulation, and the study of the regulatory mechanisms that activate the distinct osmoregulatory pathways.

At the molecular level, biophysical analysis of the K^+ channel encoded by KAT1 is currently extending our knowledge of the mechanics of inward K^+ channel function. It will be of interest to extend these molecular approaches to include the identification of proteins with which the KAT1 polypeptide interacts[20]. This will enable characterization of the immediate upstream players in the various signal transduction chains. Recent identification of an outward $^{K+}$ channel of guard cells now makes parallel studies possible for this channel type as well[108].

At the cellular level, analysis of the role of cell turgor or cell volume signals vis-à-vis regulation of the three types of K^+ channels (inward, outward, and stretch-activated) is currently lacking. Intercellular signaling and K^+ flux regulation is another intriguing topic. For example, it has been known since the 1970s that guard cell and subsidiary cell K^+ levels in the maize stomatal complex are inversely related[66]. Do guard cells signal subsidiary cells to take up K^+ during stomatal closure, or do subsidiary cells send the reverse signal to the guard cells? New techniques allowing patch clamping of guard cells in epidermal peels[34, 35] may provide an approach to address this question.

An understanding of the functional distinction between K^+ and sucrose osmoregulation will be important to understanding the interactions between pathways and their significance in terms of stomatal function. One of the most important prerequisites of these studies will be the identification of the source of osmotic sucrose in guard cells: uptake, photosynthesis, and/or starch degradation.

At the whole leaf level, given the extensive information now available on ABA regulation of ion channels in guard cell protoplasts, it would be of interest to document how ABA affects levels of guard cell K^+ and sucrose in epidermal peels and intact leaves. Identification of additional stomatal function mutants and rigorous quantification of mutant phenotypes by both physiological techniques such as gas exchange and electrophysiological techniques such as patch clamping should yield new insights into the mechanisms of guard cell solute regulation. In particular, given data suggesting that K^+ plays only a transient role as an osmoticum over the daily timecourse of stomatal opening, the signal transduction pathways that initiate K^+ efflux yet at the same time maintain stomatal apertures through increased organic solute production/uptake need to be identified. Finally, it is important to note that despite established evidence for K^+'s role as an osmoticum, we still do not have a good estimate for K^+ ion concentrations in the guard cell cytosol (as opposed to the vacuole). Further development of the tools and techniques for measuring cytosolic K^+ levels in plant cells[109] will aid in quantification of this parameter, which is of paramount importance in determining the driving force for passive K^+ flux across the guard cell plasma membrane.

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Chapter 7:
Patterns of Potassium Compartmentation in Plant Cells as Revealed by Microelectrodes and Microsampling

Roger A. Leigh[1], David J. Walker[1], Wieland Fricke[2], A. Deri Tomos[2], and Anthony J. Miller[1]

[1]Biochemistry and Physiology Department, IACR-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, United Kingdom
[2]Ysgol Gwyddorau Biolegol, Prifysgol Cymru Bangor, Bangor, Gwynedd LL57 2UW, United Kingdom

Introduction

Understanding how K^+ is distributed both within and between plant cells is important if the physiological basis of its agronomic effects are to be elucidated in detail. However, obtaining accurate measurements of K^+ concentrations in cells or subcellular compartments is difficult, and the results can be unreliable. Two recent methodological developments, triple-barrelled ion selective microelectrodes[52] and single-cell sampling and analysis (SiCSA)[49], have improved the opportunities for making measurements of K^+ in cells. These promise to increase substantially our knowledge of how K^+ and other ions behave at this level. In this chapter we briefly describe these methods and give some examples, with particular reference to K^+, of the type of information that each method can provide.

Ion Selective Microelectrodes Principles

The principle of ion-selective microelectrodes should be familiar to anyone who has ever used a pH electrode. The voltage measured across an appropriately ion-selective membrane responds to changes in the concentration or activity of the ion on one side of the membrane. After suitable calibration, the voltage output can be directly converted to appropriate units of concentration or activity. For making intracellular measurements, the electrodes must be miniaturized so that they can be inserted into cells without causing damage that could affect the veracity of the measurements. The general methods for the production of such microelectrodes have been described in detail elsewhere[2, 37, 48]. At their simplest, electrodes used for intracellular measurements must have two barrels; one containing the ion-selective membrane and the other measuring membrane potential. A measurement of membrane potential is needed because, when inserted into a cell, the barrel containing the ion-selective membrane responds to both the ion concentration/activity and the membrane potential. Thus its voltage output is the sum of the two, and a separate measure of the membrane potential is needed to obtain the response due just to the ion concentration/activity. Microelectrodes for use in plant cells must also be able to withstand the effects of turgor which can displace the ion-selective membrane from the electrode tip. This can be prevented by incorporating PVC into the membrane[37]. Ion selective microelectrodes have been used in many studies in plants and have provided measurements of the intracellular concentrations/ activities of H^+ [12], K^+ [34, 40], Cl^- [25], Ca^{2+} [38], and NO_3^- [39, 54].

One problem with using double-barreled microlectrodes in plant cells is determining whether measurements are from the vacuole or cytosol because the location of the tip cannot be visualized directly. For some ions, such as H^+ or Ca^{2+}, this is not a problem because the concentrations of these ions are different in the vacuole and cytosol, so the results fall into two populations that can be readily attributed to one compartment or the other[38]. However, for ions like K^+, where vacuolar and cytosolic concentrations are expected to be similar under some conditions, e.g., in moderately K^+-replete cells (**Figure 1**), the two populations may not be readily distinguishable. To overcome this, some independent means of assessing the location of the electrode tip is needed. This can be achieved by incorporating a third barrel which measures pH[52] and using the pH measurement to determine the electrode's location (5.0-5.5 for the vacuole, 7.0-7.5 for the cytosol)[27]. These triple-barrelled microelectrodes thus allow unequivocal measurements of ion concentrations /activities in the two major subcellular compartments of plant cells. They also have the added advantage that the simultaneous measurements of ion concentration/activity, pH and membrane potential allow thermodynamic calculations of the need for, and direction of, active ion transport at the plasma membrane and tonoplast, and an assessment of the possible mechanisms involved[39, 51].

Measurement of Cytosolic and Vacuolar K^+ Activities

Potassium occurs in both the cytosol and vacuole of plant cells. In the vacuole it has only an osmotic role. Although K^+ salts seem to be preferred as vacuolar osmotica, any other solute can potentially fulfil this role. Potassium is also an important osmoticum in the cytosol but in this compartment it also has biochemical roles for which other ions cannot substitute[31, 53]. As a result of these differences in the function of K^+ in the two compartments, Leigh and Wyn Jones[31] proposed that the K^+ concentration in each pool responds differently to K^+ deficiency and they linked this to the way plant growth changes in response to changes in K^+ supply (Chapter 2). They suggested that as K^+ concentration in the whole plant declined, the K^+ concentration in the cytosol would remain relatively constant to maintain the rate of K^+-dependent bio-

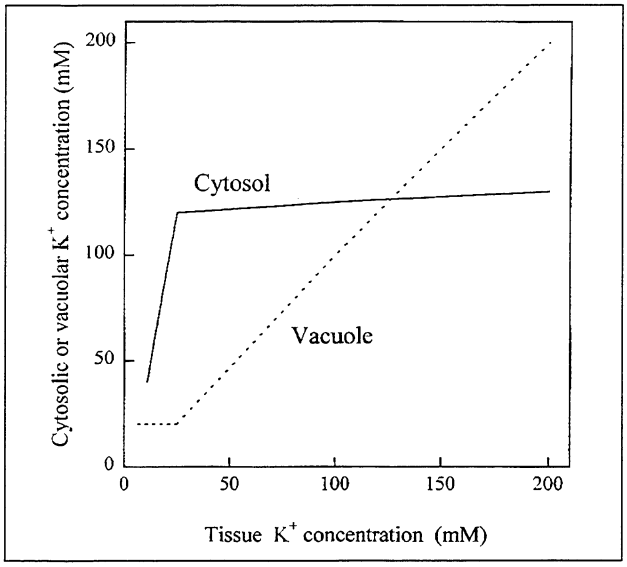

Figure 1. **Hypothetical responses of the concentrations of K^+ in the cytosol and vacuole to changes in the K^+ concentration in whole tissue (adapted from 31). A minimum vacuolar K^+ concentration of 20 mM is assumed. Once this is reached any further depletion of tissue K^+ must be at the expense of the cytosol.**

chemical processes. In contrast, K^+ concentration in the vacuole would decline, with other osmotica replacing it to maintain turgor (**Figure 1**) (31; Chapter 2). They proposed that cytosolic K^+ concentration would only decline when the vacuolar K^+ concentration had been depleted to some minimum value below which it could not fall (**Figure 1**). Thus any further change in the whole-tissue K^+ concentration would then have to be at the expense of the cytosol. Once this occurred, it would cause a decrease in the rate of K^+-dependent biochemical processes and thus lead to a decline in growth[31].

This proposed behavior of K^+ concentrations in the cytosol and vacuole is generally accepted and is backed by a variety of studies (Chapter 2). However, it has been difficult to test the idea quantitatively because the techniques available to measure compartmental K^+ concentrations have either been indirect or only semi-quantitative. Fortunately, the development and deployment of triple-barreled, K^+-selective micro-electrodes have provided a way to test the hypothesis quantitatively.

The results of a detailed study[51] on the effects of K^+ supply on the K^+ activity (a_K) in the vacuole and cytosol of barley root epidermal cells are shown in **Figure 2**. After growth in a full nutrient solution containing 5 mM K^+, the average K^+ concentration in the roots was about 150 mM while the mean a_K in vacuoles of both epidermal and cortical cells was over 100 mM and the cytosolic a_K was between 75 and 83 mM. When the K^+ concentration in the nutrient solution was decreased, there was a decline in the whole-root K^+ concentration which was matched by a proportionate and linear decline in vacuolar a_K in both epidermal and cortical cells. In contrast, cytosolic a_K remained relatively constant until the whole-root K^+ concentration had declined to about 25 mM and then it fell, but the extent of the decrease was different in epidermal and cortical cells. In epidermal cells, cytosolic a_K declined markedly and was only 39 mM in cells grown for 16 days in a nutrient solution containing 2 mM K^+. In contrast, cortical cells grown under the same conditions had a cytosolic a_K of 63 mM (**Figure 2**). Growing the roots in a solution containing only $CaSO_4$ did not significantly affect the way vacuolar and cytosolic a_K behaved (**Figure 2**). This behavior of vacuolar and cytosolic a_K is generally consistent with the model shown in **Figure 1**[31].

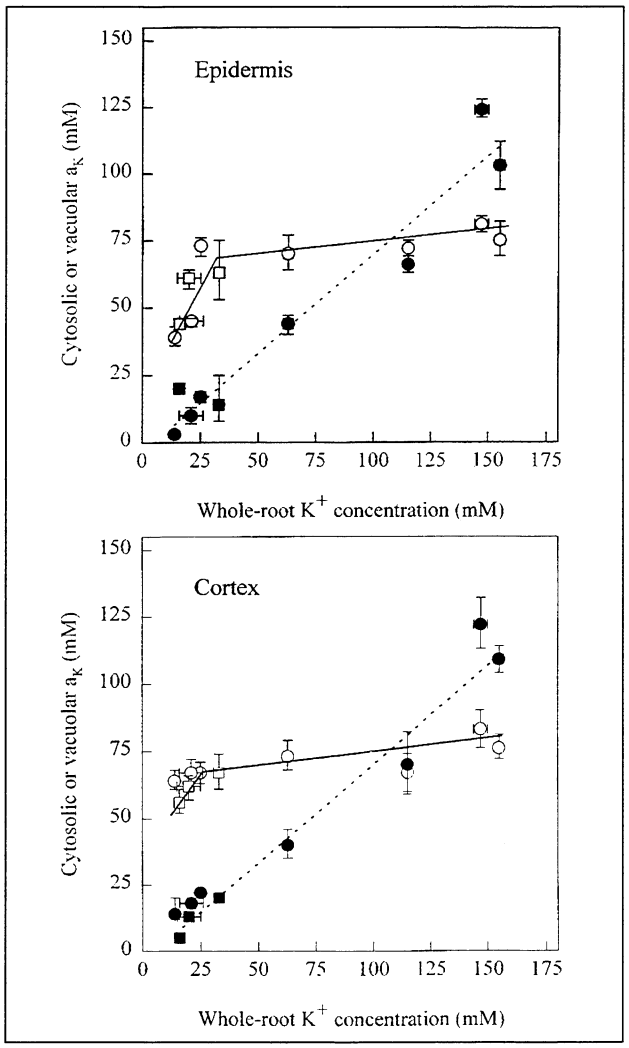

Figure 2. **The relationship between whole-root K^+ concentration and the K^+ activity (a_K) in the vacuole (closed symbols) or cytosol (open symbols) of epidermal and cortical cells of barley roots. The plants were grown in a full nutrient solution with a range of K^+ concentrations (circles) or in $CaSO_4$ (squares). Values of a_K within the cytosol and vacuole were determined with triple-barrelled, K^+-selective microelectrodes[52]. The bars show the SE for the measurement of tissue K^+ concentration, and 95 percent confidence limits for the a_K. (Adapted from 51.)**

The trigger for the decline in cytosolic a_K, particularly in epidermal cells, remains unclear. In the original hypothesis, it was proposed that it was due to the vacuolar K^+ concentration reaching a minimum value of about 20 mM, below which it would not decline (**Figure 1**). Although X-ray microanalysis studies have apparently confirmed this lower limit for vacuolar K^+ concentration[23], the triple-barreled microelectrode measurements (**Figure 2**) do not support it. Thus some factor other than the inability to mobilize K^+ from the vacuole must be responsible for causing the decline in cytosolic a_K. The identity of this factor remains to be established.

Effects of K^+ Deficiency on K^+ Transport and Cytosolic pH

As mentioned above, an advantage of using the triple-barreled microelectrodes is that they provide simultaneous measurements of compartmental a_K, pH, and membrane potential. Thus they yield information on how these parameters change with K^+ deficiency, and this can be used to assess how K^+ transport at the plasma membrane and tonoplast is affected.

There have been previous calculations of the electrochemical gradients for K^+ across the plasma membrane[26, 33] and tonoplast[13] but measurements with triple-barreled microelectrodes[51] have made it possible to extend them to different cell types. For roots grown in 5 mM K^+, the values of the electrochemical potential gradient for K^+ at the plasma membrane indicate that inward transport of K^+ is passive in both epidermal and cortical cells (**Figure 3**)[51]. In contrast, active uptake of K^+ across the plasma membrane must be invoked in K^+-deficient conditions. Further calculations indicate that in both epidermal and cortical cells of barley roots, a 1:1 H^+:K^+ symport is a feasible mechanism for achieving this, in agreement with the conclusions of others[26, 33]

At the tonoplast, active transport of K^+ from cytosol to vacuole is necessary in both epidermal and cortical cells of roots when the vacuolar a_K is greater than or equal to cytosolic a_K (**Figure 3**)[51]. A passive mechanism for K^+ uptake into the vacuole could operate under these conditions only if the trans-tonoplast potential was between -30 and -40 mV, values much higher than those recorded[51]. In contrast, when roots are grown in 2 mM K^+, and the vacuolar a_K is considerably lower than that in the cytosol, the direction of K^+ transport across the tonoplast changes with a requirement for active transport of K^+ from the vacuole to the cytosol (**Figure 3**). Two mechanisms have been proposed for active accumulation of K^+ in the vacuole: transport *via* the tonoplast inorganic pyrophosphatase (V-PPase)[9] or by a 1:1 H^+:K^+ antiport[21]. Calculations of the free energy relationships for these mechanisms[51] show that both are feasible (**Figure 3**).

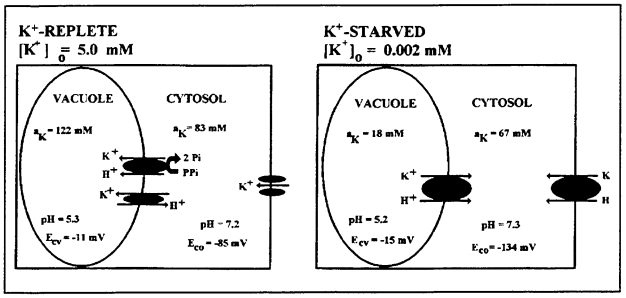

Figure 3. Potassium transport systems at the plasma membrane and tonoplast of K^+-replete and K^+-starved cortical cells of barley roots (drawn from the data in 51). In K^+-replete cells, inward transport at the plasma membrane is *via* ion channels while that into the vacuole is active. The calculations indicate that active inward transport across the tonoplast could be *via* a K^+:H^+ symport or the V-PPase[51]. In K^+-deficient cells, uptake at the plasma membrane must be active as must export from the vacuole; E_{co} and E_{cv} are the membrane potentials across the plasma membrane and tonoplast, respectively. The polarity of E_{cv} is that recommended in (3). In this convention, the potential across the tonoplast is that in the cytosol with respect to the vacuole. This results in negative values for E_{cv} which is a change from the convention that was widely adopted in the earlier literature where the trans-tonoplast potential is that in the vacuole with respect to the cytosol, i.e., a positive value. The H^+-pumps that generate the pH and electrical gradients at both the plasma membrane and tonoplast have been omitted for clarity.

In barley root epidermal and cortical cells, vacuolar pH was unaffected by K^+ supply, but K^+ deficiency did affect cytosolic pH. At cytosolic a_K values greater than 60-70 mM, the cytosolic pH in both cell types was in the range 7.2-7.4 (**Figure 4**), as expected[27]. When cytosolic a_K fell below this range, cytosolic pH decreased by 0.2 pH units for every 10 mM change in cytosolic a_K, irrespective of whether the roots were grown on full nutrient solution or $CaSO_4$. The effects on cytosolic pH were most marked in epidermal cells (**Figure 4**) where cytosolic a_K declined the most (**Figure 2**). This change in cytosolic pH was unexpected and raises the possibility that effects of K^+ deficiency on growth may be mediated through changes in pH as well as through decreases of cytosolic a_K[51]. It also raises the question of what disturbs the ability of K^+-deficient cells to regulate cytosolic pH. Three mechanisms contribute to the maintenance of cytosolic pH in plant cells[47]: physicochemical buffering[44], the biochemical pH-stat[8], and H^+ transport across the plasma membrane and tonoplast[47]. Presumably, effects on one or several of these contribute to the change in cytosolic pH in K^+-starved cells.

The cytosolic buffering capacity of plant cells is around 20 mM H^+ per pH unit[46, 47]. The decline in pH could indicate that the components of the buffering system decrease in parallel with the decline in a_K. Interestingly, K^+ deficiency also causes a decline in cell turgor of about 0.2-0.4 MPa in ma-

ture wheat root cells[42, 43]. This is equivalent to a decrease in sap osmotic pressure of about 80-160 mosmol/kg and must be matched by an equivalent change in the osmotic pressure of the cytosol. The decrease in cytosolic a_K observed in epidermal cells may be one of the components of this change, and parallel decreases in anionic species in the cytosol may contribute to the change in pH buffering capacity. It would now be interesting to measure in more detail the concurrent changes in turgor, sap osmotic pressure, cytosolic a_K, and cytosolic pH in response to K^+ deficiency.

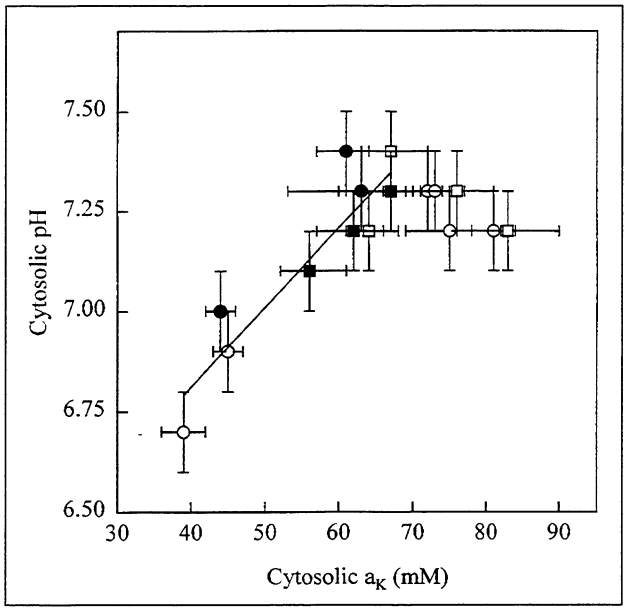

Figure 4. The relationship between cytosolic a_K and cytosolic pH in epidermal (circles) and cortical (squares) cells. Plants were grown for 2-16 days in a full nutrient solution with a range of K^+ concentrations (open symbols) or in $CaSO_4$ (closed symbols). The regression is fitted to data points for a_K < 70 mM. Bars show 95 percent confidence limits for a_K and ± S.E. for pH.

The biochemical pH-stat involves changes in the activity of malic enzyme and phosphoenolpyruvate (PEP) carboxylase in response to pH[8]. Neither PEP carboxylase nor malic enzyme are K^+-dependent enzymes[10, 11], so the capacity of the biochemical pH-stat should not depend on the K^+ status of the cytosol. Therefore, changes in this mechanism are unlikely to account for the decrease in cytosolic pH. However, the possibility of indirect effects of K^+ status on these enzymes (e.g., through expression of other regulatory mechanisms) cannot be ruled out.

If the decrease in pH is due to changes in membrane transport, it would suggest that the combined rate of H^+ influx to the cytosol and production of H^+ by metabolism are greater than the capacity of the various H^+ efflux mechanisms to remove H^+ from the cytosol. Changes in H^+ influx to the cytosol could result from increases in the activity of various transporters in response to decreases in tissue K^+ status. These would include the H^+:K^+ symporters required to transport K^+ into the cytosol across both the plasma membrane and tonoplast in K^+-deficient cells (**Figure 3**). Although active K^+ influx across the plasma membrane can also occur through a Na^+-coupled mechanism[45], this may activate other H^+-linked systems such as plasma membrane or tonoplast Na^+:H^+ antiporters which will be needed to regulate cytosolic Na^+ concentrations[5, 20, 22]. Increased activity of these will increase H^+ influx to the cytosol.

The plasma membrane H^+-pump is the major long-term regulator of cytosolic pH[47], and its activity is increased by decreases in cytosolic pH[4]. The reason why the pump should be unable to regulate cytosolic pH in K^+-starved cells is not obvious since, although it requires K^+ in its reaction cycle[6], the K^+-dependence of the enzyme is almost fully saturated at a K^+ concentration of 30 mM[32]. Thus it should be close to maximal activity at the cytosolic a_K measured in K^+-deficient cells.

Single-Cell Sampling and Analysis (SiCSA)

The SiCSA technique[49] is an elaboration of the pressure probe method developed to measure turgor in individual plant cells[24]. The approach relies on the observation that when the microcapillary of the pressure probe is inserted into a cell, a sample of sap is forced into its tip. By suitable modification of the probe, the speed at which this sample is taken can be increased so that dilution by water flow into the cell is prevented, and a representative sample of cell sap is obtained[35]. The picolitre-sized sample is stored under oil and subsamples taken for the determination of osmotic pressure and the concentrations of inorganic and organic solutes[49]. In highly vacuolate cells these cell samples are largely or entirely vacuolar in origin[36]. This has been confirmed for barley leaf epidermal cells by showing that malate dehydrogenase, a cytoplasmic marker enzyme, is undetectable in the samples[16]. However, the same study showed that this enzyme was present in mesophyll cell extracts, indicating that samples from these cells had some cytoplasmic contamination.

The advantage of the SiCSA technique is its ability to measure turgor, sap osmotic pressure, and the concentrations of major solutes, all at the resolution of single cells. Hence both water and solute relations of individual cells can be determined and the composition of different cell types analysed in detail in space and in time[15, 16, 17, 18, 19, 41].

The Solute Composition of Different Cell Types

The SiCSA technique has shown that the epidermal, mesophyll, and bundle sheath cells in barley leaves have distinctly different compositions, in confirmation of results obtained by a number of other techniques (summarized in 30). The main ionic differences between the cells are in the distribution of Pi (high in mesophyll, low in epidermis) and Ca^{2+} and Cl^- (high in epidermis low in

mesophyll), **Table 1**. When grown in nutrient solution with adequate K^+, the vacuoles of all cell types in barley leaves contain about 200 mM K^+ (**Table 1**) which is the concentration measured in whole shoot tissue or leaves of barley (e.g., 1, 28, 29). Thus all cells would appear to accumulate this ion to similar concentrations despite variations in the concentrations of other ions.

Table 1. The concentration (mM) of K^+ and other ions in epidermal, mesophyll and bundle sheath cells of barley leaves. Sap samples were taken from individual cells using the SiCSA technique. Data are given as mean ± SD and are adapted from (16.).

Ion	Epidermis	Mesophyll	Bundle sheath
K^+	216 ± 22	194 ± 41	185 ± 27
Na^+	11 ± 8	17 ± 5	16 ± 12
Ca^+	19 ± 6	2 ± 2	1 ± 1
HPO_4^{2-}	6 ± 2	61 ± 8	15 ± 11
Cl^-	76 ± 6	20 ± 5	32 ± 5
NO_3^-	228 ± 21	180 ± 30	175 ± 30

The high spatial resolution of the SiCSA technique has been used to investigate the profiles of ion concentrations within the epidermis of barley leaves and has shown that a single tissue can be heterogeneous in its solute composition[15]. In young barley leaves grown under low light intensity, the K^+ concentration was around 200 mM in all epidermal cells, although cells around the stomatal complexes and in "troughs" on the leaf surface had slightly higher concentrations than those on the "ridges" which overlay veins (**Figure 5**)[15]. However, in older leaves grown in low light there was a definite asymmetric pattern of K^+ distribution with cells between the stomata having lower concentrations than other cell types. At a higher light intensity, the pattern of K^+ concentrations in young leaves was similar to that seen in low light but it did not change so markedly with leaf age (**Figure 5**). The concentration profiles of other ions (e.g. Ca^{2+}, NO_3^- and Cl^-) were generally more variable than those for K^+ [15].

The SiCSA technique has allowed the effect of NaCl stress on the concentrations of K^+ salts in individual leaf cells to be studied. When barley plants were exposed to high concentrations of NaCl, the concentrations of Na^+ and Cl^- in the vacuoles of epidermal and mesophyll cells increased with the size of the imposed stress (**Figure 6**); see also 18. The rise in Na^+ and Cl^- concentrations in the cells was offset by a concomitant decline in the concentrations of K^+ and NO_3^-. The K^+ concentration initially declined more rapidly in mesophyll cells than in epidermal cells, but it ultimately reached a much lower value in the epidermis than in the mesophyll (**Figure 6**). At all external NaCl concentrations, the concentrations of Na^+ and Cl^- were higher in epidermis than in the mesophyll. When the stress was applied as 100 mM KCl, instead of NaCl, the K^+ concentrations in epidermal cells rose to over 400 mM[18]. In the mesophyll of these plants, the Cl^- concentration increased to 216 mM, much higher

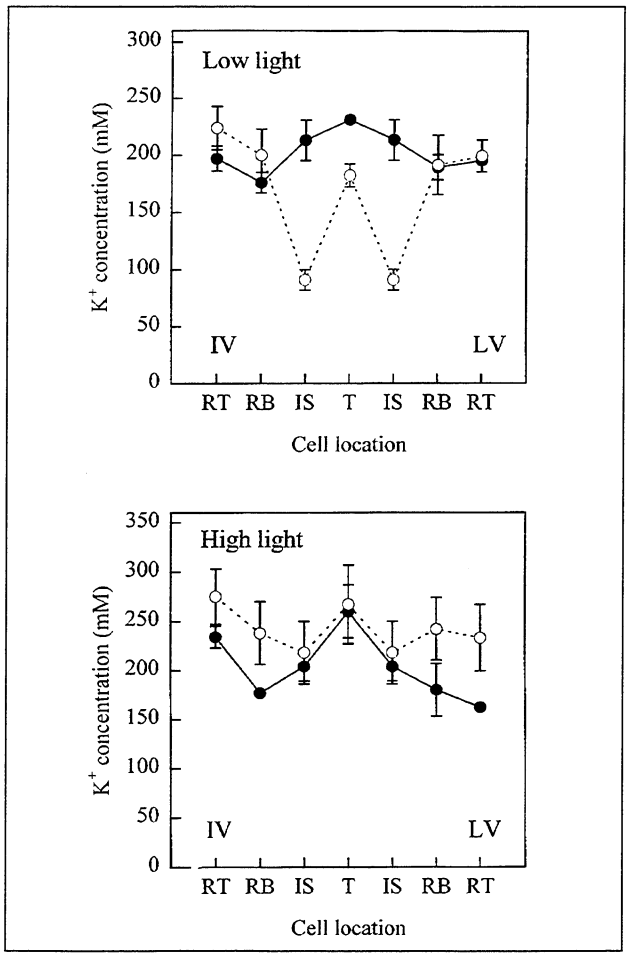

Figure 5. The concentrations of K^+ in cells in individual cells from the upper epidermis of barley leaves sampled 1 or 0 days before full leaf expansion (●) or 3 to 4 days later (○). The plants were grown in continuous light of either low (120 μmol/m²/s) or high (400 μmol/m²/s) intensity. The SiCSA technique was used to take and analyze vacuolar sap samples from files of cells located between ridges overlying the second intermediate vein (IV), counted from the midrib, to the ridge overlying the adjacent large lateral vein (LV). The cells analyzed were located on the tops of ridges (RT), at the base of ridges (RB), between stomates (IS), and in the trough between ridges (T). (The cell locations are explained in detail in 18.)

than the concentration (117 mM) reached in these cells when 100 mM NaCl was applied. The reason for this is unclear.The changes in intracellular ion concentrations resulting from growth in NaCl were accompanied by an increase in the sap osmotic pressure which, in epidermal cells, rose from 580 mosmol/kg (approximately 1.45 MPa) in control plants to 928 mosmol/kg (2.3 MPa) after growth in 150 mM NaCl. However, measurements of turgor[14] have shown that the rise in sap osmotic pressure is not matched by a concomitant increase in turgor which remains constant at about 1.0 MPa suggesting that this parameter is more closely controlled than either osmotic pressure or the concentrations of individual ions. To achieve this regulation of turgor, there must be an adjustment of the extracellular water potential in parallel with the rise in

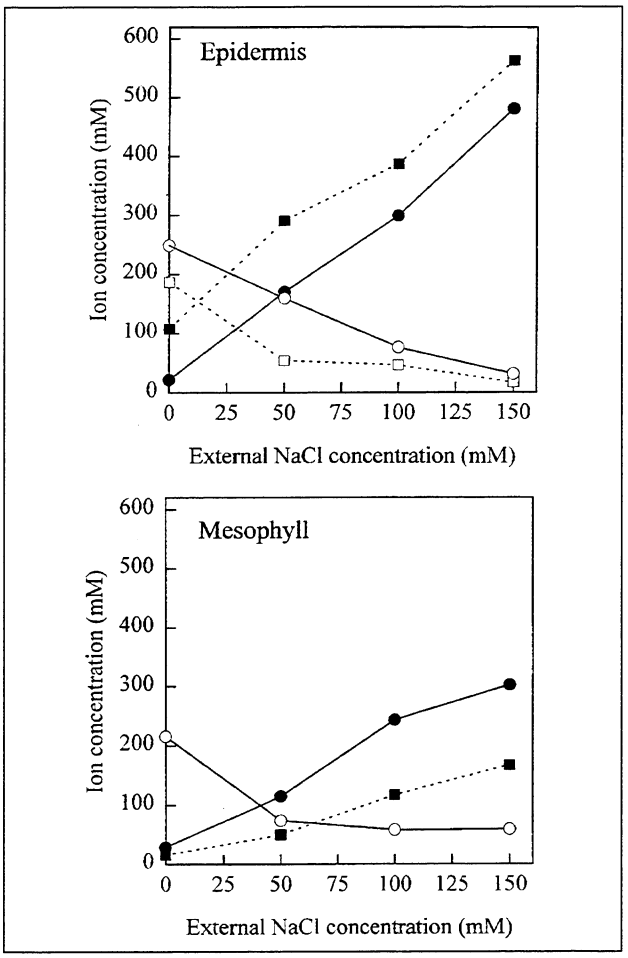

Figure 6. **Potassium (O), NO_3^- (□), Na^+ (●), and Cl^- (■) concentrations in epidermal and mesophyll cells of barley leaves grown at different external concentrations of NaCl. Sap samples were taken from individual cells using the SiCSA technique. (Drawn from the data in 19.)**

sap osmotic pressure. A similar regulation of leaf cell turgor in response to NaCl accumulation also occurs in leaf cells of the extreme halophyte, *Suaeda maritima*, and here it appears to be due to accumulation of NaCl outside the cells[7]. Regulation of turgor also occurs in cells of sugar beet storage roots as they increase sucrose concentrations during development[50]. Here the extra-cellular solute concentrations seem to be following changes in internal concentrations, not the other way around, as could be the case in salinised tissues. Therefore, there is good reason to suppose that adjustments in cell wall solutes and hence cell wall water potential are an important mechanism in turgor adjustment in response to changes in intracellular solute concentrations and that the extracellular space is a compartment in which there is active regulation and adjustment of solute concentrations. The extracellular changes that occur in barley leaves remain to be established, but it is important that they are investigated further because the ability to regulate turgor in this way could be an important aspect of salinity tolerance in this moderately halophytic crop.

As these examples indicate, the ability to determine the solute composition of cells in greater detail is demonstrating that changes in tissue composition cannot be interpreted in terms of the 'average' cell. Instead, different solutes are located in different cell types and, as a consequence, the composition of each cell type will respond differently to alterations at the whole tissue level. Hence attempts to modify the nutrient-use efficiency of plants may require a more detailed knowledge of the controls operating at the level of individual cells. Assumptions based on whole tissue analyses may be in error because the true behavior of solute pools located in different cell types is masked when such an averaging approach is used.

Summary

The contributions that developments in ion-selective microelectrodes and single cell sampling methods are making to understanding the distribution of K^+ in and between plant cells is discussed. Triple-barrelled, K^+-selective microelectrodes have provided fully quantitative measurements of the changes in K^+ activity (a_K) in the vacuole and cytosol of barley root cells grown in different K^+ concentrations. The electrodes incorporate a pH-selective barrel that allows each measurement of a_K to be assigned to either the cytosol or vacuole, based on the pH measured. The measurements made with these electrodes have revealed: (1) differences in the behavior cytosolic and vacuolar a_K to K^+ deficiency; (2) a decline in cytosolic pH in parallel with decreases in cytosolic a_K; and (3) the effect of K^+ starvation on the direction of active K^+ transport at the plasma membrane and tonoplast.

Single cell sampling and analysis have allowed the distribution of K^+ and other solutes between individual cell types to be determined. The results show that all cells in barley leaves have similar K^+ concentrations but the concentrations of other ions differ significantly among epidermal, mesophyll and bundle sheath cells. Gradients of K^+ concentration are present in the epidermis of older leaves grown in low light. Salt stress causes the K^+ and NO_3^- concentrations within barley leaf cells to decline as the Na^+ and Cl^- concentrations increase. The change in NaCl concentration in the cells is much larger than the decline in KNO_3 concentration and, as a result, osmotic pressure increases. However, turgor does not change to the same extent, indicating that this parameter is closely regulated. The studies of the intra- and intercellular distributions of K^+ and other ions are revealing new aspects of fundamental importance to elucidating the cellular mechanisms contributing to nutrient use in plants.

Synopsis of Future Research Imperatives

Several new research areas can be identified as a result of the measurements arising from the use of K^+-selective microelectrodes. First, the molecular mechanisms contributing to the regulation of

cytosolic a_K need to be identified. Transgenic plants with altered expression of K^+ transporter genes will be useful for identifying which transport processes participate in this. Second, there is a need to establish why cytosolic pH declines in K^+ deficiency. Several possible mechanisms could contribute and the relative importance of each needs to be examined. Third, the change in direction of trans-tonoplast active K^+ transport during the transition from K^+ surplus to K^+ deficiency suggests that new transporters must be synthesized; these must be future targets for cloning and characterization. Furthermore, the active transport process for mobilizing K^+ out of the vacuole in K^+ deficient cells could be characterized by patch clamp studies as this transporter must be highly electrogenic. Finally, the trigger for the decline in cytosolic a_K, particularly in epidermal cells, needs to be identified. The microelectrode measurements suggest that some factor other than the ability to mobilize vacuolar K^+ must be responsible.

The SiCSA measurements of the intercellular distribution of K^+ and other ions also suggest new research opportunities. The differences in the composition of different cell types require an explanation. They could be due either to differences in the way different nutrients reach each cell type or to the selectivity of transport systems possessed by different cells[30]. Both patch clamping and radiotracer studies with isolated protoplasts could be used to examine this. The factors contributing to turgor regulation in response to salinity and how the processes of loss of K^+ salts is controlled in response to the accumulation of NaCl also need to be examined.

Acknowledgments

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Chapter 8:
Salinity—Potassium Interactions in Crop Plants

André Läuchli

Department of Land, Air and Water Resources, University of California, Davis, CA 95616-8627

Introduction

In many areas of the world, plants can experience saline conditions. This is particularly true in arid and semiarid climate regions and regions where irrigation agriculture is dominant. Although a significant number of plant species, the halophytes, tolerate salinity and evolved specific mechanisms of salt tolerance[21], most crop plants are sensitive to salinity and poorly equipped to cope with this stress without suffering from impaired growth and development or even injury[30]. Saline environments cause plant responses through three principal effects, i.e., osmotic effects, ion toxicity, and interference with the acquisition and utilization of mineral nutrients[21, 30]. Initially, salinity causes primarily osmotic effects[21, 39], whereas after longer periods of exposure to salinity or when exposed to high levels of salt, crop plants usually are not able to regulate the flux of salt to the leaves, and the resulting salt-buildup in the plant leads to ion toxicity[21, 30, 39]. In addition, many crop plants show impaired uptake and transport of essential mineral nutrients such as K, N, P, Ca and others[21, 30].

Potassium is a critical mineral nutrient in plants, and K homeostasis, particularly in the cytoplasmic compartment of plant cells, is very important[34]. Sodium, one of the dominant ions in saline environments, interferes with K acquisition[30]. However, Na cannot substitute for K in plants, except for its use as an osmotically active ion in the vacuoles[16]. This chapter will focus on the interactions of high Na in saline environments with K in crop plants and will also emphasize the important role that Ca plays in maintaining normal K nutrition under salinity[27, 29, 30, 31]. That K acquisition is a critical process for salt tolerance in crop plants has been dramatically demonstrated by the isolation of the SOS1 mutant of *Arabidopsis* which is hypersensitive to salt, is also defective in high-affinity K uptake, and becomes K deficient[57].

Interactions at the Plant, Cell and Cell Compartment Levels

In general crop plants show decreased K concentration when exposed to salinity. This can lead to K deficiency in the plant, particularly at relatively low K supply[3]. In parallel with the lowered internal K level, Na builds up in the plant, though to varying extents depending on whether the respective crop species does or does not have Na excluding properties. As a consequence, K/Na ratios in the plant, indicative of the degree of K/Na-selectivity, frequently decrease under salinity. An example is demonstrated in **Figure 1** where it is shown that in field-grown cotton, K/Na ratios in the leaf blades and petioles decreased considerably with increasing level of salinity in the irrigation water. Decreased K in the shoot of salt-stressed crop plants is the consequence of inhibition of K influx and net uptake of K by the root (e.g. 10, 36).

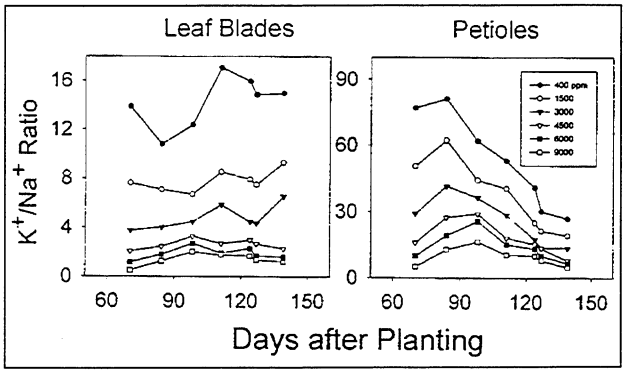

Figure 1. Effect of irrigation water salinity on K/Na ratio in leaf blades and petioles of cotton. Numbers in insert refer to irrigation water salinity expressed as parts per million (ppm) total dissolved solids. Field experiment in the Tulare Lake Basin of the San Joaquin Valley, California, 1987. (From Goyal, Sharma, Rains, and Läuchli, unpublished).

Several studies involving genetically related species or genotypes indicate the importance of salt tolerance for maintaining normal K and Na relations in the plant. The cultivated salt-sensitive tomato, *Lycopersicon esculentum* failed at high Na concentrations but was tolerant of high K while the salt-tolerant *L. cheesmanii* showed the opposite behavior: high Na tolerance, but toxic response to high K[44]. In a related study, the relatively salt-tolerant wild tomato *L. pimpinellifolium* responded to short-term salinity by increased K uptake, in contrast to the cultivated tomato[23]. This differential behavior relative to maintenance of adequate K uptake disappeared, however, when the two species were exposed to longer term salinity[23]. In different pigeonpea genotypes, K concentration in the salt-tolerant species increased with salinity but decreased in the sensitive species[55]. And the wild, salt-tolerant barley species (*Hordeum jubatum*) maintained more favorable K/Na ratios under salinity than the cultivated *H. vulgare*[56]. The latter authors suggested that the two barley species differed in their membrane properties relative to K and Na transport and selectivity.

Maintenance of intracellular K compartmentation under salinity has not been given adequate attention, but it was suggested to be of general importance for nutrient compartmentation[33]. For example, in barley leaves it was demonstrated that the vacuolar K and Na distribution profiles were altered by moderate salinity, and at higher salt levels K was no longer detectable in epidermal vacuoles while Na accumulated to high concentrations[17]. The K nutrition of the shoot meristem of lettuce was shown to be disturbed soon after salinization[32]. This effect may have a negative impact on the emergence of leaves and their subsequent growth.

The influence of salinity on subcellular ion compartmentation has gained more attention, although methodological difficulties in ion localization still pose a considerable problem. In leaves, salt may initially accumulate in the apoplast and cause a water deficit (see 54 for recent review). By means of X-ray microanalysis, the sum of K + Na was shown to accumulate to high concentrations in leaves of salinized rice plants[15]. Salt treatment of pea (salt-sensitive) and spinach (salt-tolerant) led to decreased K concentrations in cytoplasm and vacuoles of leaves of both plants without clear effects on apoplastic K concentrations[53]. As emphasized before, cytoplasmic K homeostasis[34] is crucial for normal function of plants under salt stress[40]. Homeostatic concentrations in the cytoplasm are considered to be in the range of 100-200 mM K but only 1-10 mM Na[40]. In addition, buildup of cytoplasmic Na to toxic concentrations must be avoided[54]. In salt-sensitive plants, however, the scant direct evidence available seems to indicate that Na may reach toxic cytoplasmic concentrations[54]. Relevant are data obtained from the two maize varieties LG_{11} (salt sensitive) and Protador (less salt sensitive)[24]. The results from this study (**Table 1**) indicate that the lower salt sensitivity of Protador is related to higher K concentration and K/Na ratios and lower Na concentration in the root. In a comparative study on roots of sorghum (salt sensitive) and the two salt tolerant species *Puccinellia peisonis* and *Spartina townsendii*, moderate salt stress was shown to cause a decrease in cytoplasmic K concentration in sorghum but not in *Puccinellia* and *Spartina*[28]. It appears that cytoplasmic K homeostasis may be disturbed in salt sensitive plants under salinity which would cause metabolic disorders.

Table 1. Cytoplasmic K and Na concentrations (mM) in roots of two maize varieties differing in salt sensitivity [24].

	LG_{11} (salt sensitive)		Protador (less salt sensitive)	
Ion	Control	NaCl (50 mM)	Control	NaCl (50 mM)
Na	—	142 ± 10	—	79 ± 3
K	196 ± 18	70 ± 10	175 ± 18	120 ± 26
K / Na		0.49		1.52

Interactions at Membranes

The mechanisms of K uptake by plant roots are quite well understood. The primary mechanism for high-affinity uptake from low external K concentrations is by K^+/H^+ symport at the plasma membrane[37, 40], and low-affinity uptake from external K levels in the millimolar range is down the electrochemical K gradient and occurs via inward rectifying channels[26, 40]. The driving force for the K^+/H^+ symporters and K channels is provided by the H^+-ATPase at the plasma membrane[4]. An additional high affinity K uptake transporter HKT1 has been identified from wheat roots[46] and is proposed to function as a K^+/Na^+ symport[19]. Net K uptake also involves outward rectifying channels that mediate K efflux[26].

Fundamental aspects of control of ion transport across plant cell membranes under salinity have been recently reviewed[35]. With regard to K, its influx into plant roots has been demonstrated to be inhibited significantly and increasingly with increasing salinity[10, 36]. For the wheat root HKT1 K transporter, it has been proposed that under salinity, Na binds at the K coupling site of the transporter, leading to low affinity Na uptake while K uptake is blocked[19].

It is well established that growth of salt-sensitive plants is particularly sensitive to high Na/Ca ratios in the external medium and that supplemental Ca can mitigate this detrimental effect (reviews: 29, 30). High Na/Ca ratios in the medium can cause reductions in K concentration of roots[6, 7, 42, 59] and over time of exposure may lead to K loss from the root[6]. Since Na uptake is enhanced at high Na/Ca ratios, there is impaired K/Na selectivity, particularly in the apical region of the root tip[59], but supplemental Ca can prevent this loss of K/Na selectivity[29, 59]. Parallel to the loss of K from roots exposed to high Na/Ca ratios, decreases in the root were also determined for several P-solutes (total P, Pi, glucose-6-P), probably as a consequence of increased permeability of the plasma membrane[6]. What could be the mechanism of Na-induced permeability increase of the plasma membrane? By means of a micro-fluorometric assay using chlorotetracycline as a probe for membrane-associated Ca in intact cotton root hairs, high Na was found to displace Ca from membrane binding sites, with concomitant increase in K efflux (**Figure 2**)[9]. Supplemental Ca mitigated these effects[9]. Calcium displacement by Na at low external Ca was postulated to occur from a high affinity Ca-binding site at the plasma membrane, possibly a protein[8] which would result in dramatic K efflux. At higher Ca levels, the high affinity Ca-binding site would be protected and Ca displacement would only occur from less specific, non-protein sites[8]. More recently, these findings were confirmed on plasma membrane vesicles of melon roots[58]. The following ion binding coefficients were determined:

$$K_{Na} = 0.8+/-0.2/M$$
$$K_{Mg} = 9+/-3/M$$
$$K_{Ca} = 50+/-10/M$$

It was concluded that under non-saline conditions, Ca preferentially binds to nonphospholipid binding sites of the plasma membrane. Under salinity, high Na concentration displaces Ca from these binding sites, leading to a change in membrane properties, particularly ion permeability[27,58]. This would explain the enhanced K efflux and loss of K/Na-selectivity at high Na/Ca ratios in the medium[9,59] and is also in line with the finding of greater membrane depolarization determined at high Na/Ca ratios in comparison with protection of the membrane potential at elevated Ca supply[31].

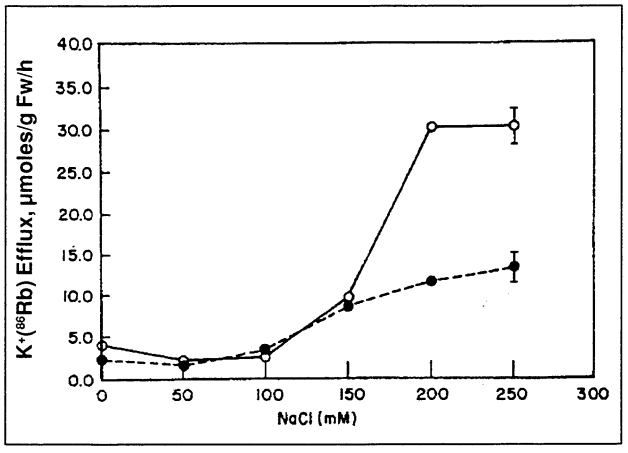

Figure 2. Interaction of Na and Ca on K efflux rate from the cytoplasm of root cells of intact cotton seedlings. o——o 0.4 mM Ca; •——• 10 mM Ca[9].

Integration with Growth and Development

To integrate quantitatively the processes that govern ion relations with growth and development of the plant, spatial and temporal patterns in growing tissues must be considered[51,52]. It is important to investigate ion distributions in growing tissues and on the temporal and spatial scales of growth distribution[52]. In addition, determination of the distribution of growth velocity will permit the calculation of ion deposition rates spatially and through time[52]. This approach has led to a detailed understanding of the growth of maize roots at low water potentials[50] and of the role K plays in osmotic adjustment of the root to low water potential[49]. A similar study was subsequently undertaken on cotton roots under salinity[59]. To calculate the K deposition rates the following equation was used[59]:

$$D = \frac{\partial S}{\partial t} + v_z \frac{\partial S}{\partial z} + S \frac{\partial v_z}{\partial z}$$

where D is the local K deposition rate, S is the density of K (amount of K per unit of root length), t is time, z is distance from root apex, and v_z is the local rate of longitudinal displacement from the root apex due to growth. This equation is based upon the continuity equation (see 51, 52). The resulting K deposition rates are shown in **Figure 3**. K deposition rates were reduced by high salt at normal Ca supply throughout the growth zone (~10 mm). Supplemental Ca, however, restored K deposition rates to control values, but only in the apical 2.5 mm region. Supplemental Ca also restored K/Na ratios in the root apex to control levels[59]. It was concluded from this study that supplemental Ca overcomes root growth inhibition by high salt through maintaining K/Na selectivity of the plasma membrane[59].

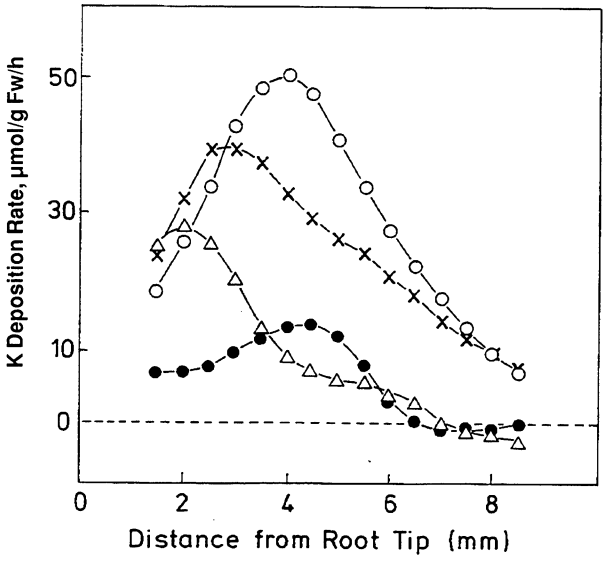

Figure 3. Spatial distribution of K deposition rates in the apical region of cotton roots[59].
o——o control (1 mM Na, 1 mM Ca)
•——• 150 mM Na, 1 mM Ca
x——x 1 mM Na, 10 mM Ca
Δ——Δ 150 mM Na, 10 mM Ca

A similar approach was used to gain an understanding of sorghum leaf growth and development under salinity and the potential role of K in these processes[1,2]. As shown in **Figure 4B**, K concentration was reduced by high salt treatment throughout the growth zone of about 30 mm, when expressed on a dry weight basis. On a fresh weight (**Figure 4A**) or leaf water weight basis (**Figure 4A**, insert), however, K concentration was also reduced by salt in most of the growth zone but exceeded control levels just beyond the growth zone. The latter effect may be interpreted to mean that K can serve as an osmoticum at early stages of leaf development under salinity[2].

Notwithstanding the importance of osmotic homeostasis for plant growth under saline conditions, more research needs to go towards determining whether reduced growth under salinity is caused more by Na toxicity or K deficiency.

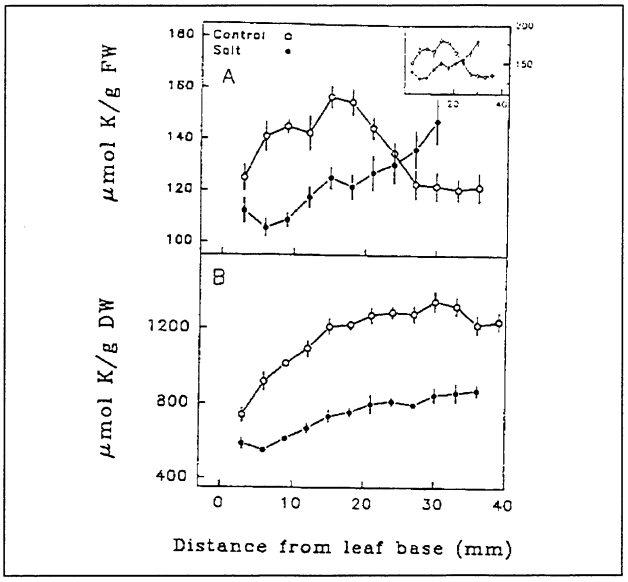

Figure 4. Spatial distribution of K concentration per gram fresh weight (A), per gram water weight (A, insert), and per gram dry weight (B) in an elongating leaf of sorghum[2].
o——o control (1 mM Na)
•——• 100 mM Na

Genetic Regulation of K/Na-Selectivity under Salinity

Progress has been made in identifying genes related to salt tolerance in crop plants, specifically relative to discrimination between K and Na[21]. In the ancestors of modern bread wheat enhanced K/Na-selectivity was first observed and located in the D genome[48]. A gene (or genes) located on the long arm of chromosome D in the D genome enhanced the wheat plant's ability to discriminate between Na and K, expressed as lower Na and higher K accumulation in the shoot[22]. Potassium/Na-discrimination in wheat at low salinities was associated with the gene Kna-1 on chromosome 4D, but this gene may be less important in the regulation of K/Na-selectivity at higher salinity[21] .

Very interesting research has been conducted in the laboratory of J. Dvorak. Tall wheatgrass (*Lophopyrum elongatum*) is remarkably tolerant of saline conditions[38]. It is phylogenetically related to cultivated wheats and is a potential source of genes for wheat improvement. Dvorak and co-workers demonstrated that an amphiploid between *L. elongatum* and bread wheat, *Triticum aestivum*, expressed salt tolerance[13]. Several chromosomes of *L. elongatum* were shown to impart significant degrees of salt tolerance in the genetic background of wheat[12]. The enhanced salt tolerance in the amphiploid is associated with a low rate of Na accumulation and maintenance of K levels in young leaf tissues[5, 41, 45]. Salt stress in the early stages caused an increase in accumulation of several ESI (early salt-stress induced) mRNAs; interestingly, this effect was less pronounced at high external Ca levels[18]. Dvorak's work is an excellent example of the use of crosses between crop species and wild relatives as sources of germplasm for improved salt tolerance, and it highlights the importance of maintaining K/Na-selectivity in young, growing tissues.

Very illuminating were recent genetic studies of salt tolerance in yeast mutants (review: 47). A mutant lacking the TRK1 gene showed greater salt sensitivity and less K/Na-selectivity than cells that contained the TRK1 gene[25]. In addition, the two yeast genes HAL1[20] and HAL3[14] were shown to greatly improve K/Na-selectivity and salt tolerance when overexpressed. The stimulation of K uptake under salinity conferred by these genes appears to be an important determinant of salt tolerance in yeast[47]. These identified mechanisms of genetic regulation of salt tolerance in yeast may serve as models for similar regulatory mechanisms in higher plants[25, 47].

It has already been mentioned that the gene HKT1 from wheat roots encodes a membrane transport protein that has the properties of the high-affinity K transporter[46]. It also mediates low-affinity Na uptake. Under salinity, high-affinity K uptake through this gene is inhibited[19, 43]. This explains the known perturbation of K/Na-selectivity in salt-sensitive plants exposed to salinity. The SOS1 gene from *Arabidopsis* which lacks a functional high-affinity K uptake system and is hypersensitive to salinity appears to encode a transport protein similar to HKT1, as far as K uptake is concerned[57]. However, in contrast to HKT1, SOS1 mutation shows also an impaired low affinity Na uptake[11]. The impaired low affinity Na uptake is not surprising in light of the findings by Gassman et al.[19] that HKT1 also functions as low affinity pathway for Na uptake. Thus, a number of genes have now been identified and characterized which encode membrane transport proteins that regulate K/Na-selectivity in relation to salt tolerance. It now appears possible to attempt to transfer such genes to crop plants in order to produce transgenic crop plants with improved salt tolerance.

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Chapter 9:
Effect of Potassium Nutrition on Carbohydrate and Protein Metabolism in Alfalfa Roots

J.J. Volenec, B.C. Joern, L. Rong, and S.M. Cunningham

Department of Agronomy, Purdue University, West Lafayette, IN 47907-1150

Introduction

Adequate potassium (K) is essential for high forage yield and persistence of alfalfa (*Medicago sativa* L.). The goals of this paper are: 1) to briefly review the impact of K nutrition on alfalfa yield and persistence; and 2) to examine the impact of K on root carbohydrate and protein metabolism. We focus on the influence of K on root metabolism because alfalfa roots are the major storage organs for starch, which serves both as an energy source and as a substrate for shoot growth in spring and regrowth after defoliation[12, 13, 29, 30]. In addition, recent studies have shown that alfalfa root nitrogen (N) also is mobilized to regrowing shoots[1, 3, 16, 17, 18, 26]. Shoot removal reduces root N uptake and nodule nitrogenase activity, leaving root and crown N as the primary sources of N during early shoot regrowth[3, 19, 32]. Partitioning of these organic reserves between roots and shoots is influenced by management and the environment, including defoliation and plant K status.

Potassium Nutrition Influences Alfalfa Performance

Studies over the last four decades have consistently shown a positive response of alfalfa forage yields to K applications when plants are grown in K-deficient soils (**Figure 1**). Yield was enhanced as K application increased from 0 to approximately 250 kg K/ha/yr. Over this range forage yield increased between 50 percent[31] and 360 percent[2]. High K is especially important for stimulating early herbage regrowth after defoliation. Kimbrough et al.[20] reported incremental increases in leaf area indices (LAIs) on day 18 of regrowth as K application increased from 0 to 372 kg K/ha (**Figure 2**). The influence of K on LAI was less apparent 32 and 39 days after harvest as LAIs of plants receiving 46, 92, and 186 kg K/ha increased to values approaching that of plants provided 372 kg K/ha. Plants not provided K had the lowest LAIs throughout regrowth. Previous work has shown that rapid leaf area expansion is closely associated with mass per shoot[33], which in turn, is a key determinant of forage yield in competitive stands[34].

Alfalfa persistence also is influenced markedly by K nutrition. Gerwig and Ahlgren[11] and Smith[31] reported improved long-term persistence (measured as percent of stand) as K applications increased from 0 to approximately 250 kg K/ha/yr (**Figure 3**). Over this range of K applications, persistence improved up to four-fold. Alfalfa persistence is influenced by both K rate and stand age. Markus and Battle[24] reported that percent alfalfa declined rapidly between Years 1 and 7 in plots provided 0 or 47 kg K/ha/yr, while it remained unchanged between Years 3 and 9 for plots provided 187 kg K/ha/yr or more (**Figure 4**). At least 93 kg K/ha/yr was needed for alfalfa to survive beyond Year 7.

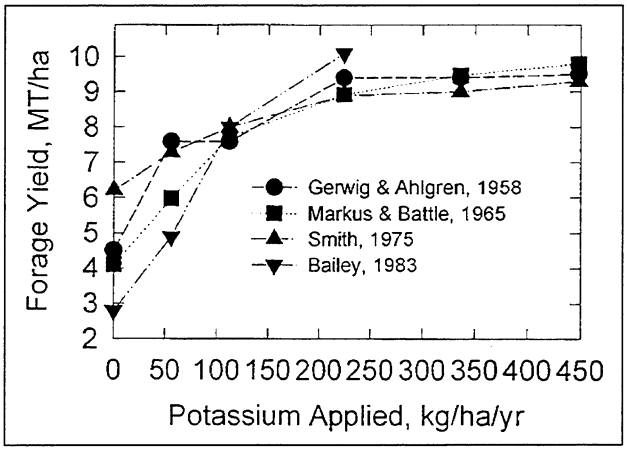

Figure 1. **Influence of K application rate on alfalfa forage yield. Data are adapted from previous published studies by Gerwig and Ahlgren (1958), Markus and Battle (1965), Smith (1975), and Bailey (1983).**

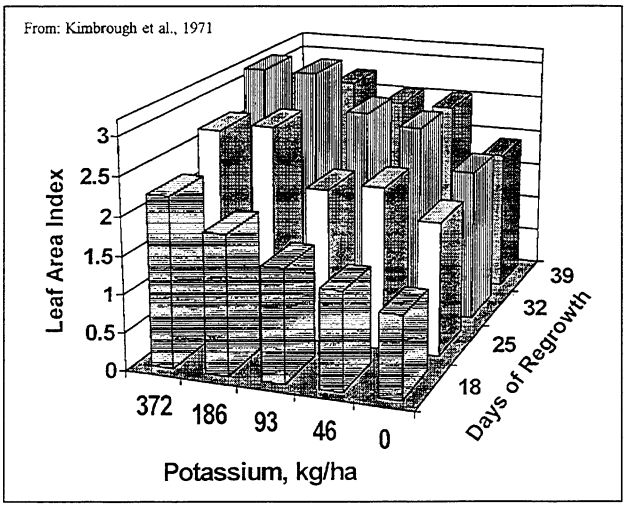

Figure 2. **Changes in leaf area index as influenced by rate of applied K and days of regrowth since defoliation. Adapted from data of Kimbrough et al. (1971).**

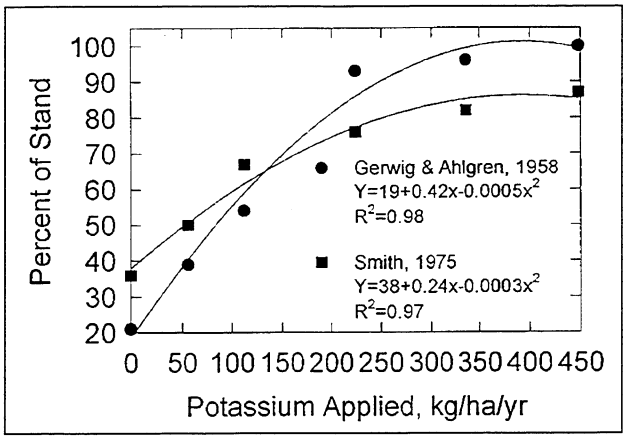

Figure 3. **Influence of K application on alfalfa persistence. Data are adapted from Gerwig and Ahlgren (1958) and Smith (1975).**

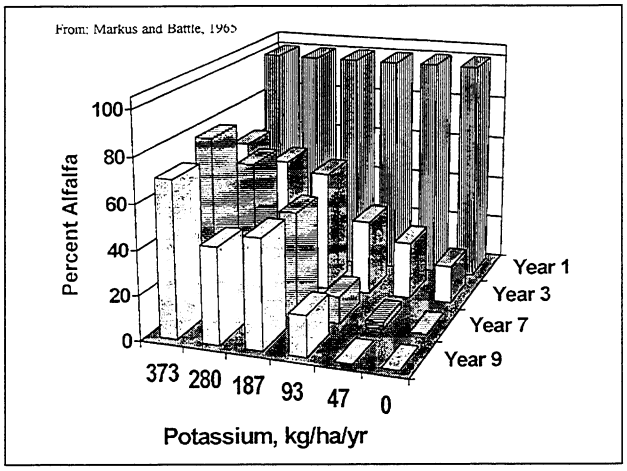

Figure 4. **Percent alfalfa as influenced by K application and stand age. Data are adapted from Markus and Battle (1965), and are expressed as a percent of Year 1 data.**

Potassium and Alfalfa Physiology

A clear understanding of how K influences alfalfa physiology and biochemistry, and ultimately improves performance, would aid alfalfa management decisions. Rhykerd and Overdahl[28] indicated that K affects a number of important plant processes including: synthesis and translocation of carbohydrates; N metabolism and protein synthesis; electrical balance of organic acids; enzyme activation; growth of meristems; and stomatal control. Results from several studies focusing on carbon (C) and N assimilation agree with their synopsis.

Net photosynthesis increases with tissue K levels up to 15 to 20 mg/g dry wt.[8, 38]. High K results in leaves with more stomata and larger stomatal apertures. Adequate K also lowers CO_2 compensation concentrations and results in greater efficiency of CO_2 fixation. Alleviation of K deficiency also has been shown to increase carbohydrate assimilation and transport to storage organs[8, 25, 27]. Wang et al.[37] found that K deficiency resulted in low concentrations of nonreducing sugars in alfalfa roots at some harvests in autumn. Root starch concentrations were not affected by K nutrition in these studies. However, plant survival was much lower in the K-deficient plots. Kitchen et al.[21] also reported increased root total nonstructural carbohydrates (TNC), the sum of sugars and starch concentrations, with K application, especially the first 100 mg K/kg soil. These authors suggested that the low root TNC caused by low K could reduce plant vigor and stand longevity. However, other researchers have observed no effect of K on root carbohydrate accumulation. Barta[4] reported no influence of K (0.1 mM to 3 mM) on root or nodule TNC concentrations. Likewise, Duke et al.[10] found that root sugar and TNC concentrations were not affected by K fertility even though low K reduced photosynthesis expressed on a plant dry mass basis[7].

Dinitrogen fixation and root N metabolism are often altered by K fertility. Collins and Duke[7] observed significant increases in N_2 fixation as K applications increased from 0 to 448 kg/ha. They attributed the low N_2 fixation rates under low K conditions to fewer nodules per plant. Surprisingly, although N_2 fixation was reduced with low K, root and shoot N concentrations were not influenced by K application. Similar results were reported by Duke et al.[10]. They found lower rates of N_2 fixation and fewer nodules on plants provided with 0 K, but found no effect of K on N or TNC concentrations of roots. They attributed the 40 percent increase in forage yield under high K conditions to increased nodulation and N_2 fixation. Barta[4] also reported that N_2 fixation was lower 10 and 14 days after harvest for plants provided 0.1 mM K when compared to those provided 0.4 and 3 mM K. This reduction was manifested as less root N/pot for plants provided 0.1 mM K when compared to those provided 0.4 and 3 mM K. Wang et al.[37] examined the influence of K on root N metabolism in autumn as plants hardened for winter. Although root crude protein (N x 6.25) concentrations were not affected by K fertility in autumn, water-soluble protein concentrations were lower in crowns of low K plants. These low K plants experienced a 50 percent mortality rate compared to a 20 percent mortality rate for high K plants, leading these authors to conclude that high K results in high crown protein levels and good winterhardiness.

We recently examined the impact of K nutrition on alfalfa root physiology because of the emerging importance of root N pools, and specifically vegetative storage proteins (VSPs), in herbage regrowth of this species[1, 3, 16, 35]. Details of the methods used in our studies have been published elsewhere[23]. Root K concentrations are easily modified by altering K levels of the nutrient solutions (**Figure 5A**), but unlike organic reserves, root K concentrations change little after defoliation. Previous field research indicated no influence of defoliation on root K concentrations of alfalfa, birdsfoot trefoil (*Lotus corniculatus* L.), sweetclover (*Melilotus officinalis* L.), or red clover (*Trifolium*

pratense L.)[22]. Root N concentrations were consistently lower in plants provided 0 mM K than plants provided 3 or 6 mM K (**Figure 5B**). Previous research revealed no influence of K nutrition on alfalfa root N[7, 10]. The low K levels attained in our nutrient solutions may have been more effective at inducing K deficiency and lowering root N concentrations. Root N concentrations of plants provided 3 and 6 mM K were similar at all harvests except 21 days after defoliation when plants provided 6 mM K had higher root N levels. Defoliation had no effect on root N concentrations of plants provided 0 and 6 mM K, whereas for plants provided 3 mM K, root N increased between days 0 and 7, then declined between days 7 and 14. Alfalfa root N concentrations are often reduced in response to defoliation[12, 22], but not always[3].

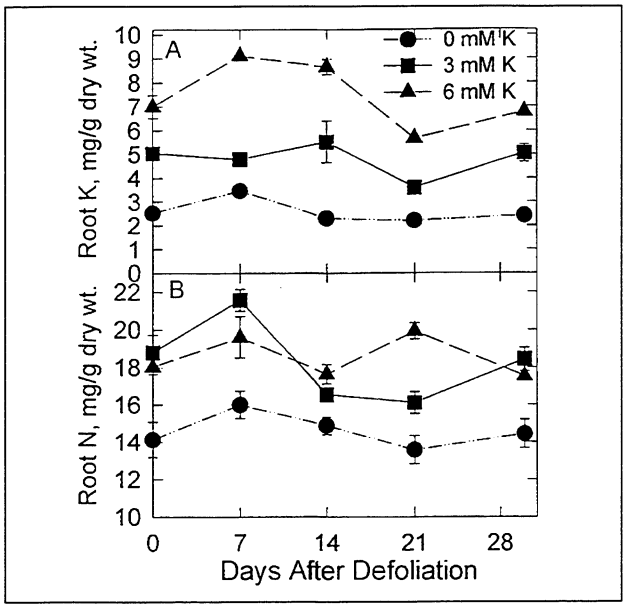

Figure 5. Concentrations of K and N in alfalfa roots as influenced by K nutrition and time after defoliation. One standard error of the mean is shown where larger than the symbol.

As expected, shoot regrowth of plants provided 0 mM K was slower than that of plants provided 3 and 6 mM K. Shoots per plant 7 days after defoliation were two-fold greater in plants provided 3 and 6 mM K when compared to plants provided 0 mM K (**Figure 6A**). By day 30 plants had approximately 2.5 shoots/plant irrespective of K treatment. Mass per shoot of plants provided 3 or 6 mM K was similar at each harvest and was 50 percent greater than that of plants provided 0 mM K beginning day 14 (**Figure 6B**). Kitchen et al.[21] also reported that reduced herbage yield of low K plants was manifested primarily as reduced weight per shoot, with little effect on final shoot number per plant.

Root TNC is thought to supply regrowing shoots with energy and substrates necessary for rapid shoot regrowth after defoliation. Root TNC levels on day 0 were proportional to K nutrition, but these differences disappeared on day 7 as TNC concentrations declined in roots of plants provided 3 and

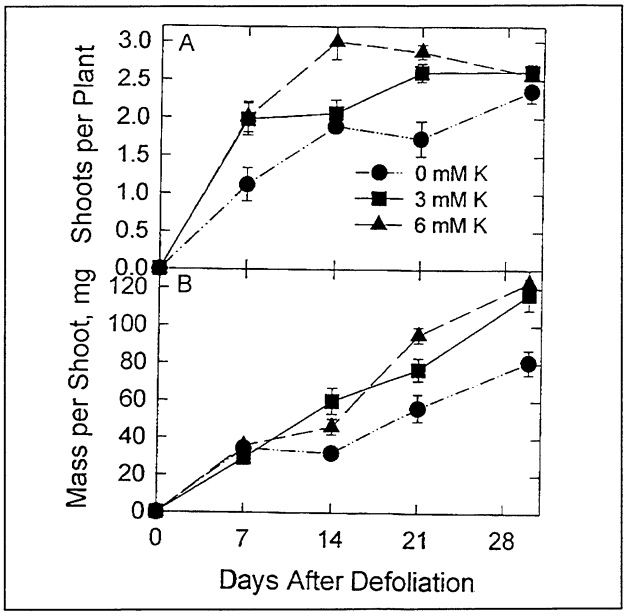

Figure 6. Alfalfa yield components as influenced by nutrition and time after defoliation. One standard error of the mean is shown where larger than the symbol.

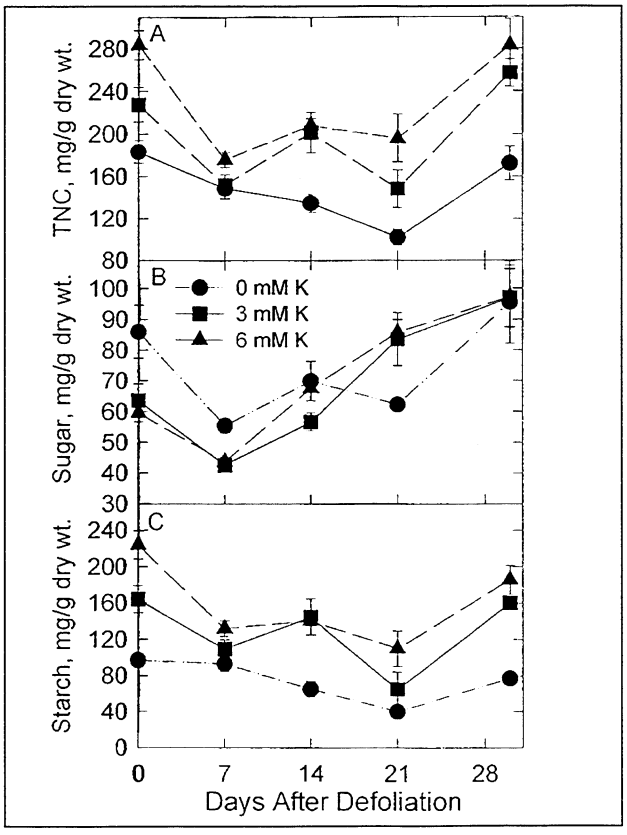

Figure 7. Concentrations of total nonstructural carbohydrates (TNC, A), sugars (B), and starch (C), in alfalfa roots as influenced by K nutrition and time after defoliation. One standard error of the mean is shown where larger than the symbol.

6 mM K (**Figure 7A**). Root TNC concentrations of plants provided 3 and 6 mM K did not change between days 7 and 14, while that of plants provided 0 mM K declined gradually. For all K treatments,

root TNC concentrations increased between days 21 and 30 attaining levels similar to those observed on day 0. Root sugar concentrations declined between days 0 and 7 irrespective of K treatment (**Figure 7B**). Root sugar levels increased thereafter for plants provided 3 and 6 mM K, while root sugar concentrations did not increase until day 30 in plants provided 0 mM K. Roots of plants provided 0 mM K had greater sugar concentrations on days 0 and 7 when compared to plants provided 3 and 6 mM K. Conversely, root starch concentrations of plants provided 6 mM K were always greater than those of plants provided 0 mM K, with the 3 mM K treatment intermediate at most harvests. For plants provided 3 and 6 mM K root starch concentrations declined from day 0 to 7, then remained relatively unchanged between days 7 and 21 before increasing on day 30. In contrast, root starch concentrations of plants provided 0 mM K were unchanged between days 0 and 7, then declined until day 21 before increasing on day 30. Root starch concentrations observed on day 30 were similar to those observed on day 0 for all K treatments. Barta[4] and Duke et al.[10] reported no effect of K on root TNC or sugar concentrations, whereas Kitchen et al.[21] found a significant increase in root TNC as K application increased from 0 to 50 mg K/kg soil.

We examined root soluble protein concentrations because recent evidence suggests that root proteins are a significant N source for regrowing shoots after defoliation[1, 3, 26]. Root protein concentrations of plants provided 0 mM K were markedly reduced on day 0 when compared to roots of plants provided 3 and 6 mM K (**Figure 8**). Protein concentrations declined between days 0 and 14 in roots of plants provided 3 and 6 mM K, but returned to predefoliation levels on day 30. Protein levels increased slightly between days 0 and 7 in roots of plants provided 0 mM K, then declined until day 21 with no change thereafter.

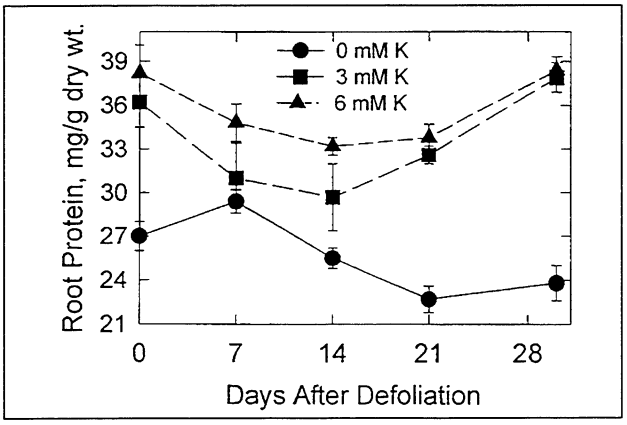

Figure 8. **Concentration of buffer-soluble protein in alfalfa roots as influenced by K nutrition and time after defoliation. One standard error of the mean is shown where larger than the symbol.**

Alfalfa roots contain several polypeptides that are preferentially degraded after defoliation and that appear to serve as vegetative storage proteins, VSPs[3, 9]. On day 0, roots of plants provided 0 mM K had lower levels of root VSPs when compared to plants provided 3 mM K (**Figure 9**; 15, 19 and 32 kD polypeptides). Defoliation on day 0 reduced VSP abundance in roots of plants provided 3 mM K when sampled on day 14, but VSPs returned to high levels by day 30. Little change in relative abundance of VSPs occurred after defoliation in roots of plants provided 0 mM K. Low K has been shown to reduce dinitrogen fixation[4, 7, 10]. However, addition of N to plants receiving 0 mM K does not increase root protein concentrations nor does it restore root VSP synthesis in alfalfa roots[23]. Application of N as nitrate had little effect on root protein and VSP accumulation, whereas application of ammonium reduced protein and VSP accumulation even if 3 mM K was provided. The basis for the interaction of N form with K nutrition that results in a reduction of root protein and, in particular, root VSP is not clear and is currently under study.

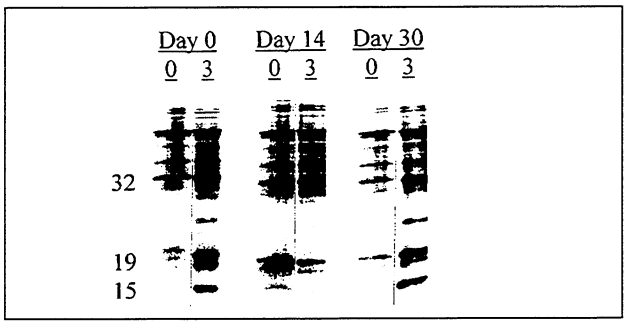

Figure 9. **Polypeptide composition of alfalfa taproot proteins determined using SDS-PAGE. Equal amounts of protein (25 µg) were analyzed per lane. Roots from plants provided 0 or 3 mM K were sampled at defoliation (day 0), and 14 and 30 days later.**

Summary and Research Imperatives

The key features for alfalfa shoot regrowth and plant persistence are located in roots and crowns since these organs remain after defoliation and over winter. Although K can have a tremendous impact on shoot regrowth and plant survival of this species (**Figures 1 to 4**), our understanding of the mechanistic role K nutrition plays in alfalfa performance is uncertain. It is generally believed that root TNC reserves are key physiological factors in alfalfa regrowth and winter survival[6, 15, 30]. The impact of low root TNC concentrations on alfalfa regrowth must be interpreted with care because changes in root TNC are often not independent of changes in other root factors, including root protein. Frequent harvesting, untimely autumn harvesting, and other mismanagement treatments are often used to deplete root TNC and result in the poor alfalfa shoot growth and plant death that are often attributed to low root TNC reserves. However, previous research using genetic selection to

alter root starch accumulation revealed little association between root starch concentration and regrowth rate or winter hardiness of alfalfa[5]. In contrast, alfalfa shoot regrowth after defoliation has been consistently associated with root N concentrations[26, 35]. In these studies, plants with low root N concentrations have slow shoot regrowth despite having abundant root TNC reserves. In addition, root N compounds can provide both C and N to regrowing shoots. Recent findings verifiy that up to 58 percent of shoot C comes from C skeletons of root N compounds[1]. Amino acids and VSPs in roots are the N pools that are most readily mobilized to regrowing shoots[3]. Low K reduced accumulation of VSPs in this (**Figure 9**) and previous studies[23].

If root starch and VSPs are essential for rapid shoot regrowth and persistence of alfalfa, it would be useful to understand the role of K in synthesis and degradation of these organic reserves. The high sugar concentrations in roots of plants provided 0 mM K (**Figure 7**) suggest that sugar availability may not limit starch synthesis in alfalfa roots under low K conditions. Hawker et al.[14] showed that in vitro activity of potato (*Solanum tuberosum* L.) starch synthase was enhanced by K addition. The role of K in stimulating activity of starch-synthesizing enzymes in roots of forage legumes has not been investigated. In addition, the role of K in protein synthesis has been previously examined in many systems. However, the mechanism by which K deficiency specifically reduces alfalfa root VSP accumulation is unknown. We are currently developing molecular tools that will enable us to examine the role of K and other factors in the transcription of VSP genes and translation of VSP messenger RNA.

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Chapter 10:
Physiological Changes during the Development of Potassium Deficiency in Cotton

C.W. Bednarz and D.M. Oosterhuis

Altheimer Laboratory, University of Arkansas, Fayetteville, AR 72701, USA.

Introduction

Widespread K deficiency has occurred across the U.S. Cotton Belt. However, the explanation for these deficiencies is unclear, and a considerable amount of research and speculation has surrounded this phenomenon. It has been postulated that K deficiency is related to earlier-maturing, higher-yielding, faster-fruiting cotton varieties creating a greater K demand than the plant root system is capable of supplying[11]. Cotton appears to be more sensitive to low K availability than most other major field crops and often shows signs of K deficiency on soils not considered to be K deficient[5].

In cotton, tissue tests have become a valuable diagnostic tool for assessing the nutrient status of a crop, for determining fertilizer recommendations during the growing season, and for detecting potential K deficiency[1,8]. The petiole is generally considered to be more indicative of plant K status than the leaf blade, partly because of the more rapid decline in K concentration in the petiole, compared to the leaf, during the boll development period[1,8]. However, there is still some question about the appropriate critical or threshold levels for K concentration in the leaf or petiole, as these values may be appreciably altered by environmental factors, plant genetics, and sampling procedure.

The objective of the studies described here was to investigate changes during the onset of K deficiency in cotton with regard to partitioning of K in plant components during K deficiency. These studies have been previously reported[2,3,11].

Materials and Methods

Cotton (cv. Deltapine 20) was germinated in 8-L pots containing sand in a growth chamber, located at the Altheimer Laboratory in Fayetteville, AR, with a photo-period of 12 hours, photosynthetic photon flux density of 980 µmol/m^2/s, day/night temperature of 30/25°C, and day/night relative humidity of 50/80 percent. Plants were watered daily with deionized water and with nutrient solution on alternate days. The nutrient solution contained 14 mM NO_3, 2 mM NH_4, 6 mM K, 4 mM Ca, 2 mM P, 2 mM S, 2 mM Mg, and micronutrients. In the K deficient treatment, K was withheld from the nutrient solution used by replacing 6 mM KNO_3 with 3 mM NH_4NO_3. In the K deficient treatment, K was withheld from the nutrient solution used by replacing 6 mM KNO_3 with 3 mM NH_4NO_3. At 14 days after planting (the fourth true leaf stage) two treatments were established consisting of (1) continued complete nutrient solution, and (2) nutrient solution containing no K. Samples were taken 13, 19, and 26 days later and measurements made of organ K concentration, plant growth parameters, leaf chlorophyll, photosynthesis, ATP, and nonstructural carbohydrate concentrations as plant K deficiencies developed. Details of techniques and specific procedures are given in Bednarz[2].

Results and Discussion

Significant reductions in tissue K concentration in the no-K treatment were observed in all organs on each analysis date when compared to the plus-K treatment (**Figure 1**). Petiole K showed the highest concentration in the plus-K treatment, while leaf K showed the lowest concentration. All organ K concentrations in the no-K treatment were less than 10 g/kg at 19 and 26 days after withholding K. Large numerical differences were observed at 19 and 26 days in leaf area, leaf dry weight, root dry weight, and square dry weight (data not shown), but only on day 26 were some significant differences (P=0.05) observed. The sensitivity of

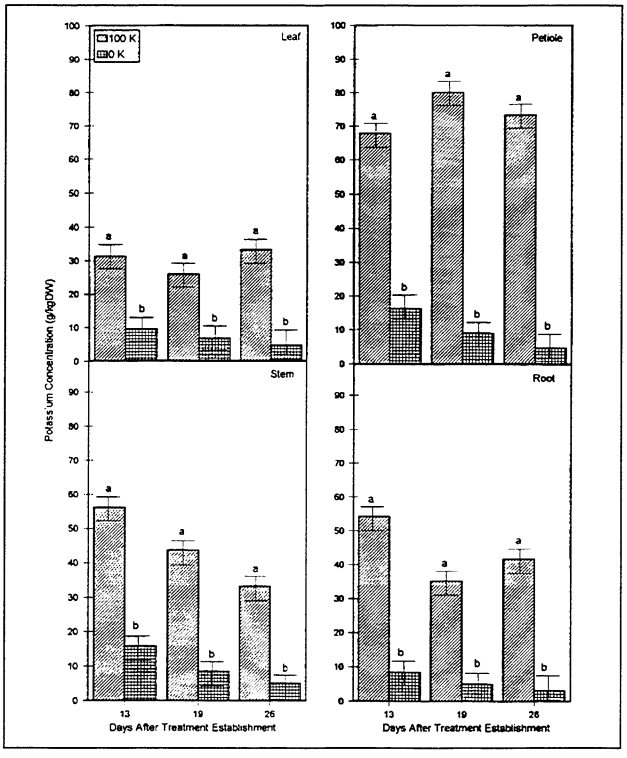

Figure 1. Leaf, petiole, stem and root K concentrations at 13, 19, and 26 days after withholding K. Vertical bars (±SE) within an organ and date followed by the same letter are not significantly different (P=0.05).

various cotton organs to K deficiency (in terms of decrease in K accumulation) was: bolls < stems and petioles < leaves < roots, such that bolls were the last organs to be affected. This is in contradiction to the order of sensitivity reported by Rosolem and Mikkelsen[12].

There were no visual K deficiency symptoms 13 days after withholding K, and leaf chlorophyll concentrations were similar in both treatments (data not shown). However, 19 days after withholding K, slight margin and interveinal chlorosis was observed in the leaves. Chlorophyll *a* and total-leaf chlorophyll concentrations from the no-K treatment were significantly lower on this day. By 26 days after withholding K, severe chlorosis, as well as the beginning of necrotic areas, was observed in the tagged leaves, as is typical for K deficiency[11]. Reductions in chlorophyll were also observed in the no-K treatment, along with a reduction in the chlorophyll *a* to chlorophyll *b* ratio (C*a*:C*b*), indicating reductions in chlorophyll *a* were occurring faster than reductions in chlorophyll *b*. Various stages of visual leaf K deficiency symptoms were observed in all leaves of the canopy by 26 days after withholding K.

Treatment differences in leaf photosynthesis were not observed until 19 days after withholding K when leaf photosynthesis was significantly reduced by 80 percent in the no-K treatment and by 95 percent at 26 days (**Figure 2**). The critical leaf K concentration has been reported to occur between 1.2 and 0.9 percent[1]. Yet, our data show leaf photosynthesis did not begin to decline until leaf K concentration fell below 0.95 percent and petiole K concentration fell below 0.88 percent. Also, leaf photosynthesis increased from 13 to 26 days after withholding K.

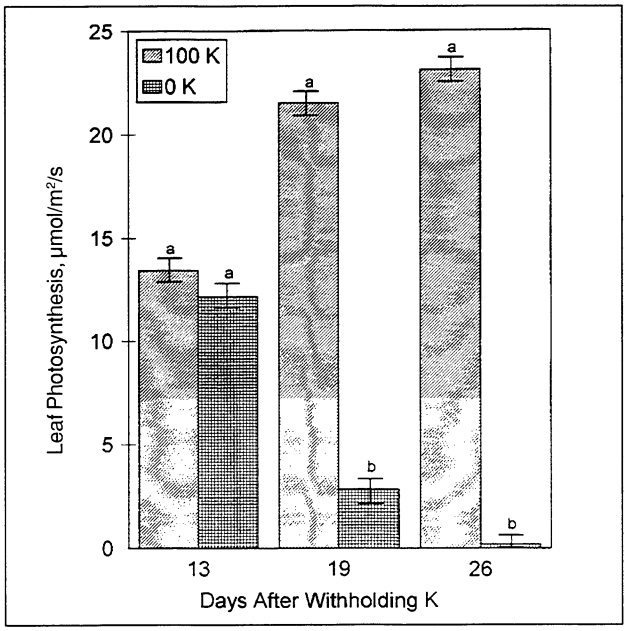

Figure 2. Leaf photosynthesis at 13, 19 and 26 days after withholding K. Vertical bars (±SE) followed by the same letter within a date are not significantly different (P=0.05).

Some studies have suggested that K deficiency will result in reduced ATP synthesis[7]. Our results show leaf ATP concentration increased as the K deficiency became more acute in the no-K treatment at 19 and 26 days after withholding K (**Figure 3**). Therefore, ATP utilization may have been restricted more than ATP formation, which would also agree with Huber[9]. Leaf hexose (glucose and fructose) from the plus-K treatment remained fairly constant in all samples throughout the sampling period (**Figure 4**). However, at 13 days after withholding K, leaf hexose from the no-K treatment was elevated in the evening samples. Also, at 19 and 26 days after withholding K, hexose concentrations were much higher in leaves from the no-K treatment at both sampling times. Increased leaf hexose concentration may be attributed to the decreased activity of K-dependent enzymes such as pyruvate kinase[6], or from greater hydrolysis of sucrose by the increased activity of acid invertase[9] or other sucrose metabolizing enzymes. Leaf sucrose from the plus-K treatment was also fairly uniform throughout the sampling period (**Figure 4**). Leaf starch was always higher in the samples taken in the evening than in the morning, regardless of K treatment (**Figure 5**). Again leaf starch at 19 and 26 days after withholding K was much higher in the no-K samples at both sampling times. Electron micrographs of leaf cross sections confirmed the presence of starch in the no-K treatments but not in the plus-K treatments (data not shown). Finally, total leaf soluble sugars (glucose, fructose, and sucrose) followed the same trends as leaf hexose and sucrose concentrations.

In a related study, Bednarz et al.[2] showed that accompanying the decreased photosynthesis as the K deficiency developed in the no-K treatment was

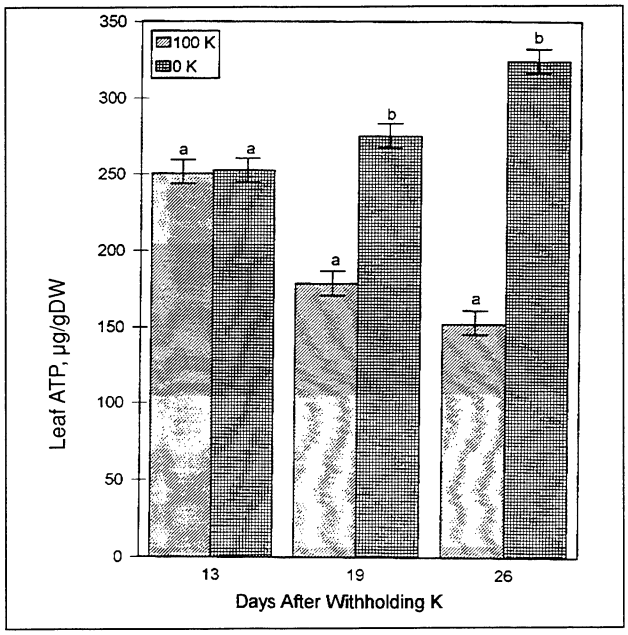

Figure 3. Leaf ATP concentrations at 13, 19 and 26 days after withholding K. Vertical bars (±SE) followed by the same letter within a date are not significantly different (P=0.05).

a decreased carboxylation efficiency and an increased CO_2 compensation point. Both gas exchange (A/Ci curve, **Figure 6**) and C isotope analyses (data not shown) showed that stomatal conductance was most limiting to photosynthesis 13 days after withholding K, whereas at 19 and 26 days non-stomatal conductances were most limiting. Most of the work involving stomatal and non-stomatal limitations of photosynthesis and isotopic fractionation in a tissue sample has concentrated on the effects resulting from plant water stress. However, the changes that occur in both the photosynthetic apparatus and the resulting C isotope composition during the development of a K deficiency in cotton have received little or no attention.

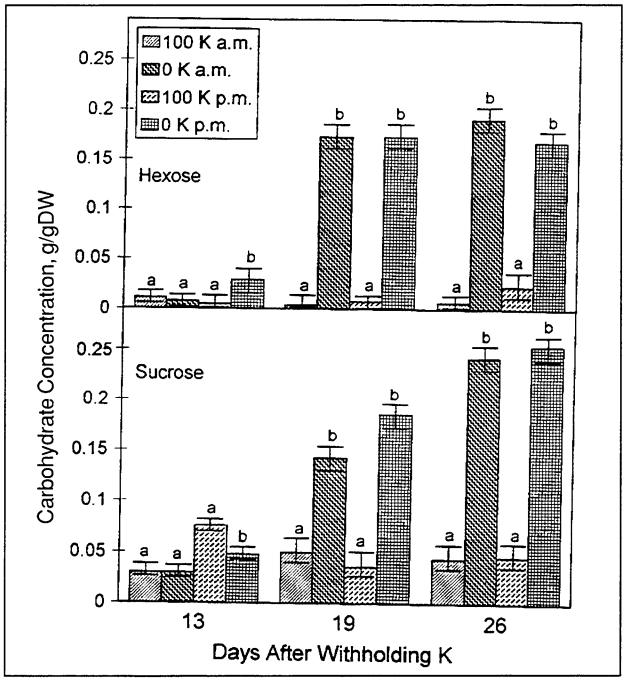

Figure 4. **Leaf hexose and sucrose concentrations at 13, 9 and 26 days after withholding K. Vertical bars (±SE) followed by the same letter within a date, sugar and time are not significantly different (P=0.05).**

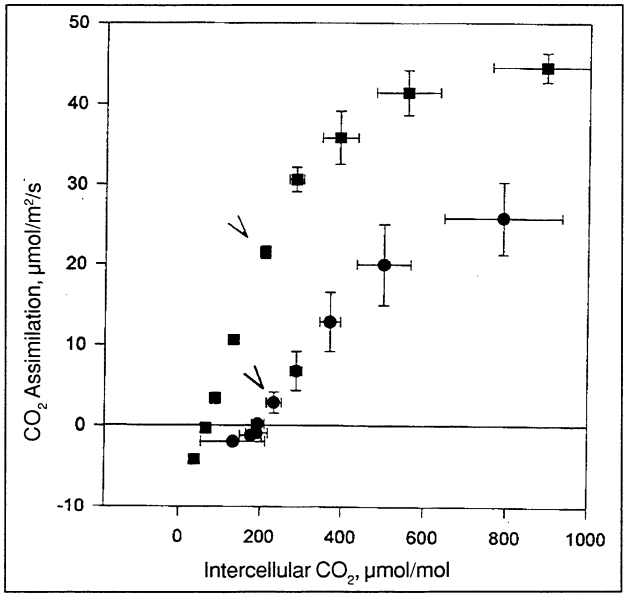

Figure 6. **Rate of CO_2 assimilation versus intercellular CO_2 concentration 19 days after withholding K. Arrows indicate the points obtained at an external CO_2 concentration of 350 µmol /mol.**

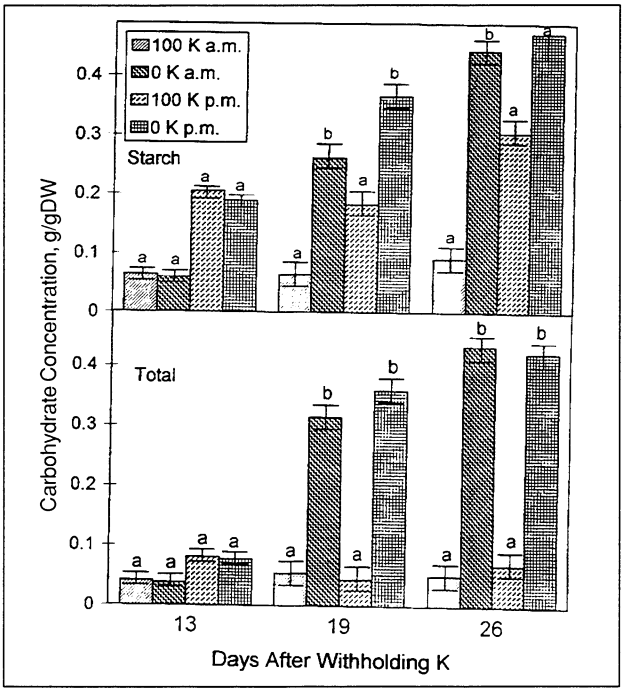

Figure 5. **Leaf starch and total soluble sugar concentrations at 13, 19 and 26 days after withholding K. Vertical bars (±SE) followed by the same letter within a date, sugar and time are not significantly different (P=0.05).**

Potassium fertility recommendations based on cotton petiole diagnostic analysis results have been inconsistent, partly because the lowest acceptable petiole K concentration is unknown. Our studies show that reductions in leaf physiological processes and plant growth did not occur until the petiole K concentration fell below 0.88 percent on a dry weight basis. Therefore, reductions in lint yield and quality should not develop until this critical petiole level is attained. These results can help to improve the efficiency of K fertilizer usage in cotton production.

Cotton plants continue to accumulate K at rates above those needed to produce maximum yields, especially prior to the reproductive stage[4], and this luxury consumption could possibly confuse tissue diagnostic recommendations[3]. However, Kafkafi[10] suggested that luxury consumption of K can be both beneficial for high yields and a cheap source of insurance against possible K deficiency problems. Oosterhuis[11] suggested that the luxury storage of K by the cotton plant may explain the apparent inability of researchers to accurately predict the onset of K deficiency from tissue analysis.

Our studies have documented the effect of an increasing K deficiency on various physiological processes and related these to tissue K concentra-

tions. The large differences observed in tissue K did not significantly affect growth and leaf physiological functions at day 13 after withholding K. The critical leaf K concentration was between 0.95 and 0.67 percent on a dry weight basis, while critical petiole K concentration was between 0.88 and 0.46 percent. After 19 days, however, most of the measured parameters were already greatly affected by the lack of K, and thus we conclude that critical leaf K concentration is closer to 0.95 percent. Also, from the large differences observed in tissue K at day 13 with no changes in leaf physiological functions, together with the larger increase in tissue K as opposed to dry matter, we conclude that K was stored in luxury amounts. Leaf chlorophyll did not begin to decline in the no-K treatment until 13 days after withholding K. The chlorophyll data coincide well with photosynthesis, which also did not begin to decline until day 13 after withholding K. Increased ATP in the no-K treatment was also observed 13 days after withholding K and probably resulted from the decreased utilization of this metabolite rather than from increased production. These findings will be useful for interpreting plant analyses and for more accurate determination of plant requirements from soil or foliar K applications[11] before a pending deficiency would decrease growth or yield.

Synopsis and Future Research Imperatives

This report describes studies conducted in Arkansas on the K nutrition of cotton. Initial studies documented the partitioning of K in plant components during the season. The onset of K deficiency in growth chamber experiments was first detected in roots, followed by stems, petioles and leaves, and then in the fruit. Furthermore, luxury storage of K prior to peak demand for K by the boll load could complicate tissue diagnostic recommendations. Visual K deficiencies were first observed in growth chamber experiments 19 days after K was withheld, along with reductions in leaf chlorophyll concentration and significant reductions in leaf photosynthesis. However, leaf ATP and nonstructural carbohydrate concentrations were higher 19 and 26 days after withholding K than in the control, which may have been the result of reduced utilization and translocation of these metabolites. Carbon isotope analyses and analysis of carbon isotope discrimination indicated that the most limiting resistance to net photosynthesis was stomatal, but that non-stomatal limitations became more important as the K stress developed. Reductions in leaf physiological processes and plant growth did not occur until the petiole K concentration fell below 0.88 percent on a dry weight basis. Therefore, reductions in lint yield and quality should not develop until this critical petiole level is attained. This information will improve the understanding of tissue diagnosis for a pending K deficiency. Results from these studies were used to explain the inconsistent response of cotton to foliar-applied K fertilizer.

Future work should explore the fate of foliar-applied K from the leaf surface into the leaf and the role of this absorbed K in metabolism and specifically in boll growth. The use of labeled K or Rb will be needed in this study.

The use of petioles lower in the canopy versus the currently sampled petiole, fourth node down from the terminal, to detect a pending K deficiency needs to be tested in the field in different locations, varying soil K levels, and crop conditions. In particular, the size of the boll load needs to be taken into consideration, as the stronger the sink for K the more the deficiency is likely to be manifested lower in the canopy in the petioles closer to the developing fruit load.

Water deficit stress is a typical and widespread problem in cotton in the U.S. Cotton Belt. However, there is only limited information on the effect of plant water status on the efficiency of leaf absorption of foliar-applied K. The uptake of foliar-applied K should also be studied in relation to environmental conditions, temperature, relative humidity, and time of day of the application.

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Chapter 11:
Foliar Potassium Fertilization of Cotton

D.M. Oosterhuis

Altheimer Laboratory, University of Arkansas, Fayetteville, AR 72701, USA.

Introduction

Speculation has surrounded the explanation for the widespread appearance of K deficiency across the U.S. Cotton Belt in recent years. The occurrence of a complex of K-deficiency symptoms in cotton was first recognized in California during the early 1960s[3, 15]. These deficiencies have manifested themselves during the latter half of the season in a range of soils and cotton cultivars. However, the explanation for these deficiencies is unclear, and a considerable amount of research and speculation has surrounded this phenomenon. It has been shown that the occurrence of K-deficiency is related to the incidence of Verticillium wilt[90]. It has also been speculated that the deficiency symptoms may be associated with the use of higher-yielding and faster-fruiting cotton cultivars and the increased use of N fertilization in cotton management[68].

Many K deficiencies can be corrected through preplant soil applications, or partially corrected using mid-season sidedress applications of K. Foliar applications of K may offer the opportunity of correcting these deficiencies more quickly and efficiently, especially late in the season when soil application of K may not be effective. Foliar applications have the advantage of allowing producers to add the necessary K when tissue analysis indicates a pending shortage, thereby correcting the deficiency and preventing yield loss.

There is a wealth of literature about foliar fertilization which was used as long ago as 1844 to correct plant chlorosis with sprays of Fe[28]. However, the practice has only caught on in cotton production in the last two decades. In 1991 it was estimated that about 9,000 tons of K fertilizer was foliar applied to cotton in the U.S. Cotton Belt. However, there is still considerable speculation about the benefits and correct implementation of this practice. While there are many reports on research involving soil-applied K[49], there are no definitive studies available on the usefulness of foliar-applied K. A report in 1976[63] indicated that foliar applications of K significantly increased seedcotton yield. There have also been more recent reports of foliar applications of K improving both lint quality and yield[70]. With the national emphasis on lint quality[82] and the introduction of high volume instrumentation classification, the positive effect of K on lint quality may be of paramount importance.

This paper provides an overview of K nutrition of cotton, the current understanding of foliar fertilization of cotton and the benefits of this practice in cotton production. The review focuses on the importance of K in plant growth, its uptake and distribution in the plant, K deficiency symptoms and causes. The review also covers the fertilization of cotton, with some emphasis on foliar fertilization and related aspects. The review concentrates on plant nutrition rather than soil nutrition and, therefore, does not cover the various aspects of soil K availability or methods of analyzing soil K content.

Importance of Potassium for Plant Growth

Potassium is an essential nutrient for all living organisms and is required in large amounts for normal plant growth and development[54]. In higher plant cytoplasm, K is the dominant cation and is commonly found to be in concentrations ranging from 80 to 150 mM[12]. It is absorbed by roots from the soil as the monovalent cation K^+ usually by active uptake. Potassium is very mobile in the plant and can be translocated against strong electrical and chemical gradients[38].

Potassium is not a constituent of any known plant components, but it is integrally involved in metabolism and plant water relations. Its primary role is as an enzyme activator. It has been implicated in over 60 enzymatic reactions[26] which are involved in many processes in the plant such as photosynthesis, respiration, carbohydrate metabolism, translocation, and protein synthesis. Potassium balances charges of anions and influences their uptake and transport. Another important function is the maintenance of osmotic potential and water uptake[25]. These two functions of K are manifest in its role in stomatal opening[45] when stomatal conductance and turgor are coupled. Another major role of K is in photosynthesis[44] by directly increasing leaf growth, leaf area index, and, therefore, CO_2 assimilation[95]. Potassium increases the outward translocation of photosynthate from the leaf[2].

There have been a number of reviews of the K nutrition of cotton (e.g. Hearn, 1981[36]; Kerby and Adams, 1985[49]). Potassium plays a particularly important role in cotton fiber development, and a shortage will result in poorer fiber quality and lowered yields[18]. Potassium is a major solute in the fiber (single cells) involved in providing the turgor pressure necessary for fiber elongation[23]. If K is in limited supply during active fiber growth, there will be a reduction in the turgor pressure of the fiber resulting in less cell elongation and shorter fibers at maturity. As K is associated with the transport of sugars, it is likely implicated with second-

ary wall deposition in fibers and, therefore, related to fiber strength and micronaire. Xi et al.[96] reported poor cuticle development in cotton plants grown without sufficient K, which may have resulted in increased water loss by non-stomatal transpiration. Potassium has been reported to reduce the incidence of Verticillium wilt[30] although the physiological reasons for this are not clear.

Potassium Uptake and Distribution in the Cotton Plant

Potassium is required in large quantities by cotton: from 3 to 5 kg K/ha/day[32, 94]. The total quantity of K taken up by the plant is related to the level of available soil and fertilizer K[10, 49]. An average mature cotton crop is estimated to contain between 110 and 250 kg/ha of K, of which about 54 percent is in the vegetative organs and 46 percent is in the reproductive organs[77]. However, only about 20 kg of K are needed to produce one bale (218 kg) of cotton fiber, with about 2.5 to 6 kg being removed mainly by the seeds[39, 77].

Plant uptake of K follows a pattern similar to dry weight accumulation (**Figure 1**), except that dry matter continued to increase until maturity, whereas maximum K accumulation was reached in about 110 days after which there was a decrease[34]. Potassium was absorbed more rapidly than dry matter was produced, as evidenced by the higher concentration of K in young plants[7]. The rate of K uptake was slow during the seedling stage, about 10 percent of the total, but increased rapidly at flowering and reached a maximum of 4.6 kg/ha/day between 72 and 84 days[32]. Mullins and Burmester[61] reported maximum daily uptake rates of 2.24-3.47 kg/ha/day 63 to 98 days after planting. Basset et al.[7] reported corresponding values of 2.1 to 3.4 kg/ha/day between 90 and 127 days after planting for older later-maturing varieties.

The need for K increases dramatically when bolls are set on the plant because bolls are a major sink for K[50]. These authors showed that the total K in an individual boll increased from 0.19 mg/boll 10 days after flowering to 1.19 mg/boll at boll maturity 56 days after flowering. This appears low compared to the 126 mg K/boll reported by White[94].If an average cotton crop contains about 150 kg K/ha[39] with about 50 percent of the K in the reproductive unit[77], then for a hypothetical average 100 bolls/m^2, there would be 75 mg K/boll. This is closer to the higher value of White[94] although his data were from Acala cotton, which with a larger boll size should have a higher total K (T.A. Kerby, personal communication).

During boll development the K concentration increased from 19 to 55 g/kg dry weight between 10 and 55 days, and K concentration in the fiber decreased from 22 to 6 g/kg at boll maturity[50]. The decline in fiber K concentration was due to redistribution of the K within the boll to the seed and capsule wall during the seventh and eighth week

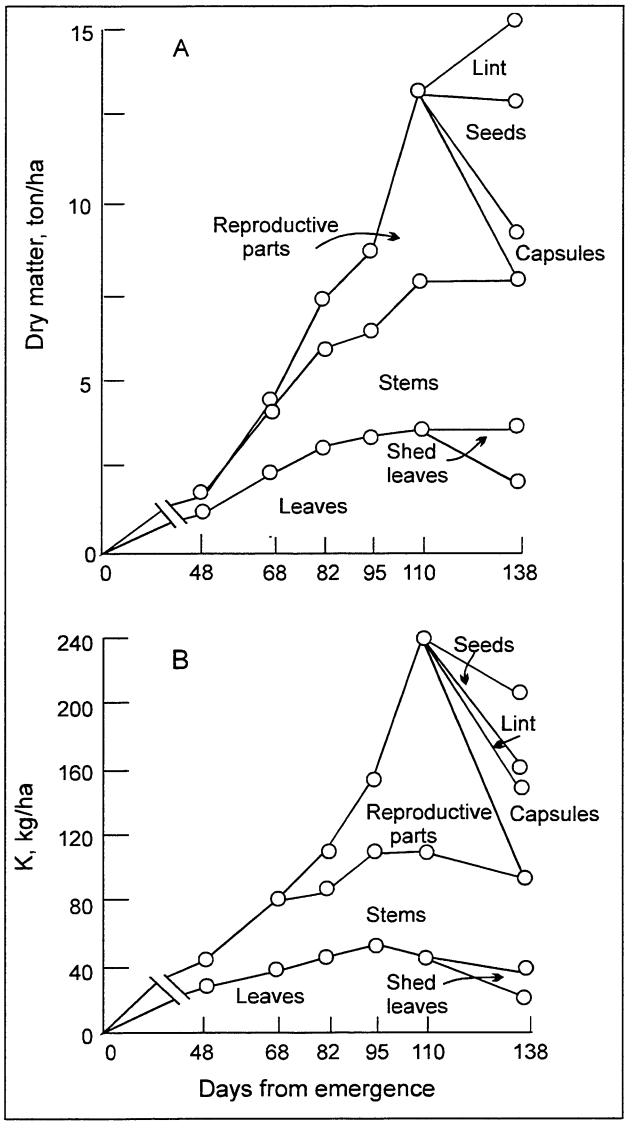

Figure 1. Accumulation and distribution of dry matter (A) and uptake and distribution of K (B) in the aerial components of the cotton plant during the season (from Halevy et al., 1987).

of boll development. The high concentration of K in the boll is related to the role of K in the maintenance of osmotic potential to generate the turgor pressure necessary for fiber elongation[23]. Potassium is the most abundant cation in cotton fiber[50]. The capsule wall of the boll contains approximately 4 percent K and accounts for between 32 to 60 percent of all the K accumulated by the boll[7, 49].

The mineral nutrients and assimilates for the growth of young leaves, the early "sinks", are translocated from the root and stem as well as from mature leaves. As fruiting begins, the developing boll load forms a new and stronger sink which becomes dominant for available assimilate[50]. During boll development, K is withdrawn from the older leaves and petioles and is retranslocated via the phloem to the bolls. This strong source-sink relationship is critical for high yields and any factor that adversely affects the relationship can result

in K deficiencies and lower yields and fiber quality.

Potassium can be taken up in luxury amounts[47], and this could possibly confuse tissue diagnostic recommendations. However, there is evidence that luxury consumption of K is actually beneficial and a relatively cheap insurance policy against environmental stress[47]. Potassium is the most abundant cation in the phloem sap[35] amounting to about 80 percent of the total cation sum. It is usually stored in the vacuoles in large quantities. Recent reports by Bednarz and Oosterhuis[8] suggested that the luxury storage of K by the cotton plant may explain the apparent inability of researchers to accurately predict the onset of K deficiency from tissue analysis.

Sensitivity of Cotton to Potassium Availability

Cotton appears to be more sensitive to low K availability than most other major field crops and often shows signs of K deficiency on soils not considered K deficient[17]. Cope[21] reported that in a 21-year field comparison of five field crops (cotton, vetch, corn, wheat, and soybeans), cotton was the most sensitive to K deficiency and was the most responsive to K fertilization. Potassium is relatively immobile in the soil and moves slowly, mainly by diffusion[6]. The rate of plant uptake of K depends on root length density and total root surface area[14]. However, the cotton root system is notable for its low density relative to other major row crops[27]. Thus, the relative sensitivity of cotton to the soil K supply may reflect in part the low density root system of the cotton plant. The high requirement for K in cotton coupled with an inherent low root length density and the immobile nature of the element means that K uptake is particularly sensitive to poor root growth, and deficiencies may appear even in soils with a relatively high K content. Furthermore, anything which restricts root growth, such as disease or insect damage, nematodes, root pruning, poor drainage, soil acidity, compaction etc, will reduce nutrient uptake and may, therefore, exacerbate K deficiency.

Symptoms of Potassium Deficiency

Potassium deficiency occurs more frequently and with greater intensity on cotton than for most other agronomic crops[49]. Typical K deficiency symptoms consist of yellowish-white mottling of the leaves that changes to numerous brown specks at the leaf tips, around margins, and between veins[85]. The leaf tip and margin curl downwards as the tissue breakdown continues. Finally, the whole leaf becomes rust colored, brittle, and drops prematurely, stopping boll development. This results in dwarfed and immature fruit, some of which may not open. Small bolls are a typical symptom of severe K deficiency in cotton. Many of these symptoms are related to the disturbance of tissue water balance resulting in tip drying, leaf edge curling, and early senescence. Potassium deficiency symptoms in cotton are quite distinctive and, due to the characteristic bronzing that occurs, were once termed *cotton rust* before the true cause was known[49]. The symptoms of K deficiency have been mistaken for Verticillium wilt symptoms as they seem to occur under similar environmental conditions[92]. Furthermore, the growth and yield of cotton varieties less susceptible to Verticillium wilt are often less affected by late-season K deficiency[3, 56].

Potassium deficiency symptoms fall into two categories, namely those that occur at the bottom of the plant on the lower, older or mature leaves, and the more recent symptoms[53, 86, 92] that show up on young cotton leaves at the top of the plant, late in the season. The characteristic rusting and premature senescence is the same for both lower and upper canopy K deficiencies. However, unlike the lower, older leaf symptoms, researchers have not been fully able to explain the real cause of these new upper-canopy deficiency symptoms which have aroused much speculation. Current thinking is that modern varieties develop bigger yields over a shorter fruiting period, and K moving upward from the roots is intercepted by the developing boll load at the expense of the upper leaves.

The sensitivity of various cotton organs to K deficiency (in terms of decrease in K accumulation) was reported by Rosolem and Mikkelsen[80] as: leaves < bolls < roots < stems. These results indicate that by the time K deficiency symptoms are manifested in the leaves, the growth of all other plant parts (including the economically important boll) are already detrimentally affected. However, a recent report by Bednarz and Oosterhuis[9] showed a contradictory order of organ sensitivity to K deficiency in cotton as: bolls < stems and petioles < leaves < roots, such that bolls were the last organ to be affected.

Reasons for the Widespread Occurrence of Potassium Deficiencies in Cotton

Much speculation has surrounded the widespread occurrence of apparent "K deficiency" symptoms that have appeared in recent times in the U.S. Cotton Belt. Many theories have been proposed to account for these deficiencies, but the explanation is still not clear. It has been proposed that the deficiencies are related to soils with K availability problems[18], the relative inefficiency of cotton at absorbing K from the soil compared to most other crop species[17], or the incidence of Verticillium wilt[90]. It has also been postulated that the widespread K deficiency that has occurred in recent years is related to earlier-maturing, higher-yielding, faster-fruiting cotton varieties creating a greater demand than the plant root system is capable of supplying[71].

The decrease in root activity after the start of flowering[16] may further exacerbate the K deficiency syndrome. This is because the decrease in root

growth occurs during peak K demand as the developing boll load increases and exerts the major demand for available assimilate, including K. When K is limiting in the soil, this decline in root activity can be expected to have a dramatic effect on K uptake by the roots and therefore, on the growth, management, yield and lint quality of the cotton crop. The high demand for K by the developing boll load will be further hindered if root development is poor due to nematodes, compaction, high water tables, or cool soils as are often experienced early-season in the Mississippi Delta. Irrigated and dryland cotton crops have different rooting patterns[11]. Dryland crops have a more extensive root system with significant genotypic differences. This could also influence the availability of K.

Recent evidence indicates that modern cotton cultivars have less K in storage prior to boll development[9] which could account for the unpredictable appearance of K deficiency in certain environments. This would be further exacerbated by the higher yields and bigger boll loads, and concomitant increase in K requirement, of modern cotton crops. In addition, in recent years more cotton has been planted on poorer soils low in available K[49].

The modern K deficiency syndrome appears to be a complex anomaly related to: (i) the greater demand for K by higher-yielding modern cultivars, (ii) the inability of the root system to supply this demand due to the decrease in root activity late in the season or due to poor or restricted root growth, (iii) soil K fixation, (iv) the relative inefficiency of cotton at absorbing K from the soil compared to most other crop species, (v) possible relationships with diseases such as Verticillium wilt, and possibly, (vi) less storage of K by modern cultivars prior to boll development. Obviously all these factors are related to environmental conditions, and influenced by production management practices.

Fertilization with Potassium

The goal of fertilizer programs for cotton should be to achieve maximum economic return for the fertilizer investment[49]. This may not necessarily coincide with maximum yield, and it may change with time and with location. Fertilizer applications are made to meet the annual crop nutrient requirements and return to the soil those nutrients removed by the crop. Adequate fertilization is important to every cotton farmer because the amounts used, and therefore the cost, are slight compared to the dollars lost from yield limitations[31].

An effective fertilizer management program must include consideration of the optimum times when the different nutrients are needed as well as the fate of the nutrients when applied to the soil. The uptake pattern for K by cotton is well documented[7, 32], with the need for K rising dramatically when the boll load begins to develop[32] because the bolls are the major sinks for this nutrient. However, most fertilizer programs utilize a single preplant application of K. This may not always be sufficient because the peak demand by the plant occurs much later during boll development, and because of the many factors that can affect K uptake by the cotton plant (the decline in root growth during boll development, nematodes, soil K fixation, etc).

A knowledge of the soil being used is important because the mineralogy, organic matter, and level of K depletion for a specific soil can significantly affect the fate and availability of applied fertilizer K[78]. Accurate soil analysis coupled with mid-season plant tissue analysis is needed to formulate a suitable K fertilizer program. Soil sampling and analytical methods of assessing soil available K are reviewed by Sabbe and Zelinski[81].

Most fertilizer applications of K are surface applied or shallowly incorporated into the topsoil. Previous research in California by Gulick et al.[29] showed that cotton root systems fail to exploit available K in the topsoil adequately. These authors suggested that K uptake by cotton could be improved if a large proportion of the root system was exposed to adequate available K. Mullins et al.[62] suggested that cotton may, therefore, respond to deep placement of K in the subsoil. They demonstrated that deep placement of about 16 kg K/ha produced higher yields than surface broadcast applications, although at higher rates the surface broadcast application consistently produced higher yields than deep placement. Research in the Mississippi Delta has shown increased yields on some soils as a result of deep placement of K fertilizer at a depth of 15 to 30 cm[88]. Deep placement of fertilizer K has not consistently resulted in yield increases (T. Keisling, personal communication) and additional research is needed. Soils exhibiting the greatest response to deep placement of K generally have subsoils with low to very low soil K.

Foliar Fertilization with Potassium

Potassium deficiencies can be corrected through preplant soil applications or partially corrected using mid-season sidedress applications of K. Foliar applications of K may offer the opportunity of correcting these deficiencies more quickly and efficiently, especially late in the season when soil application of K may not be effective or possible. Foliar applications have the advantage of allowing producers to add the necessary K when tissue analysis indicates a pending shortage, thereby correcting the deficiency and preventing yield loss. It is of interest that foliar feeding of a nutrient may actually promote root absorption of the same nutrient[87].

While there are many reports on research involving soil applied K , there are very few on the usefulness of foliar-applied K. Oosterhuis[63] working in southern Africa reported significant increases in cotton yield from foliar fertilization with K (as KNO_3) without any apparent K deficiency. Halevy and Markovitz[33] in Israel reported increased lint yield and average boll weight from foliar sprays

containing N, P, K and S in locations where the soil fertility was low.

More recent research in Arkansas[70, 71, 72] indicated that foliar-applications of KNO_3 can increase yields and improve lint quality (**Table 1**). Five treatments were used: (1) a control with no added soil or foliar K, (2) low (33.6 kg K/ha) soil-applied KCl preplant (3) high (67.2 kg K/ha) soil-applied KCl preplant (i.e. at twice soil recommendations) (4) low preplant soil-applied KCl and foliar-applied KNO_3, and (5) high preplant soil-applied KCl and foliar-applied KNO_3. The foliar treatment was applied at a rate of 11.2 kg KNO_3/ha at 2, 4, 6, and 8 weeks after first flower in 94 liters water/ha using a CO_2 backpack sprayer. In addition, 1.54 kg N/ha was added as foliar urea to treatments 2 and 3 each time the foliar-KNO_3 was applied to the foliar treatments 4 and 5 to negate the possible effect of the N in KNO_3. The effect of foliar-applied K averaged over five locations in Arkansas between 1989 to 1992 is presented in **Table 1**. The average yield increase was 73 and 17 kg lint/ha compared to the low and high soil-applied K treatments, respectively. The average lint yield was increased from 1,107 kg/ha in the untreated control to 1,136 kg/ha in the low soil-applied K treatment (using recommended levels of K), and increased to 1,207 kg/ha in the combined low soil-K plus foliar-K treatment. However, in some years the high-soil K treatment was really a "normal" recommended level[84] and the high-soil K treatment was, therefore, similar to the low-soil K plus foliar-K yield. Under those circumstances it would be difficult to justify foliar K, unless deficiencies appeared and/or petiole analysis revealed a need for additional K.

In some cases it appeared possible to achieve the same affect as the foliar K by doubling the initial soil K. This may not, however, be practical due to possible salt buildup and K fixation in some soils. Furthermore, excessive K application to the soil, on soils testing low in Mg, can induce Mg deficiency and reduce yield if fertilizer recommendations are not followed closely[22]. It is interesting that foliar K without any soil-applied K increased lint yield an average of 81 kg/ha compared to the untreated control, and 25.8 kg/ha compared to the standard soil K treatment (**Table 1**). The small response to K in certain years was probably due to the extended growing season which allowed the younger upper-canopy bolls, which would not usually have had the K or the time to fully develop, to grow into mature harvestable bolls.

Boll weight (seedcotton) was increased from 3.52 g/boll in the soil-applied KCl control plots to 3.87 g/boll in the soil plus foliar K plots[70]. As with yield, the greatest influence on boll weight was obtained from the combined soil-K plus foliar-K treatment. Potassium deficiency symptoms occurred in all treatments at most sites but least of all in the soil-plus-foliar K treatment. Petiole analysis of upper-canopy leaves indicated that the combined application of soil and foliar K significantly enhanced plant K content compared to controls during both vegetative and reproductive development. The soil test K level averaged about 345 kg K/ha...172 parts per million (ppm)...(**Table 1**). From a regression analysis of the data, yield increase can probably be expected when using foliar-applied K on soils with a relatively low soil K status of less than 125 ppm K (Oosterhuis, unpublished data), although in some cases responses to foliar fertilizer on cotton growing in soils with a higher K status have been recorded.

Fiber quality was also significantly improved by foliar applied KNO_3, with the increases occurring primarily in fiber length uniformity and strength (**Table 2**). Micronaire was also increased in certain years. Application of KNO_3 either as foliar treatments alone, or in combination with supplemental soil KCl, effectively improved uniformity and strength. Surprisingly, however, soil application of KCl alone did not enhance any of the fiber quality components. Fiber quality in 1991 was unaffected due to above average quality of the cotton crop in general. Foliar plus soil K increased fiber dry weight compared to the preplant soil applica-

Table 1. The influence of soil- and foliar-applied K on cotton lint yields at four sites in Arkansas, 1989 to 1992. (From Oosterhuis et al., 1992 and 1993).

	1989	1990			1991		
Treatment	MES[1]	CBS	MES	NEREC	MSCO	CBS	SEBES
	kg lint/ha						
Control	606 0	1579 c	834 c	982 a	1043 b	1157 a	1499 a
Low soil K	619 bc	1725 b	844 bc	1000 a	1102 bc	1148 a	1515 a
High soil K	—[3]	—	—	967 a	1133 ac	1210 a	1490 a
Foliar KNO_3	631 ab	1725 b	907 ab	—	—	—	—
Low soil + foliar K	649 a	1839 a	913 a	1020 a	1257 a	1224 a	1580 a
High soil + foliar K	—	—	—	1015 a	1222 a	1182 a	1394 a
Preplant soil K[4]	400	176	314	546	274	272	217

[1] MES = Main Experiment Station, Fayetteville; CBS = Cotton Branch Station, Marianna; NEREC = North East Research and Extension Center; MSCO = R.D. Jackson Farm, Mississippi County; SEBES = Southeast Branch Experiment Station.

[2] Values within a column followed by the same letter are not significantly different (P = 0.05).

[3] Treatment not included.

[4] Preplant 0 to 15 cm soil test K status in kg K/ha.

tion of K. The K concentration and the K content of the fibers were also increased by the foliar plus soil K application[71]. The capsule wall contained the highest amount of K of the three components and may have acted in a storage capacity.

Table 2. The influence of soil- and foliar-applied K on cotton fiber quality, as measured by high volume instrumentation (from Oosterhuis et al., 1990).

Treatment	Length uniformity index, %	Strength, g/tex
Control	84.5 b[1]	24.4 b
Soil-applied KCl	85.8 b	24.2 b
Foliar-applied KCl	87.1 a	26.6 a
Soil- + foliar-applied KNO_3	86.0 ab	25.1 ab

[1]Values within a column followed by the same letter are not significantly different (P=0.05).

A three-year Beltwide study from 1991 to 1993 evaluated the effect of foliar-applied KNO_3 compared to soil-applied KCl on cotton yield and fiber quality[73]. The study was a cooperative effort conducted under different environmental conditions at 12 sites from North Carolina to California. The preliminary results have been variable with significant yield increases from foliar K recorded about 40 percent of the time[67].

Research findings to date suggest that where a potential K deficiency exists, KNO_3 applied as a foliar spray to supplement preplant soil-applied K can have a significant effect on cotton yield and fiber quality. However, more information is needed on the underlying physiological explanation of cotton K requirements and K deficiencies to better predict the need for, and the response to, foliar application of K.

Foliar Fertilization of Cotton Seedlings with Potassium

Adverse environmental conditions are often experienced in the Mississippi Delta during seedling development. Producers have, therefore, become interested in foliar fertilization of seedlings to enhance their growth during this critical stage. However, little is known about the benefits of foliar fertilization of cotton seedlings, even though foliar application of urea or B to cotton during flowering is a widely used practice to enhance boll development. Field research at five sites in Alabama showed that cotton yield was not influenced at all by the application of foliar N-P_2O_5-K_2O (12-48-8) fertilizer made in one to three applications at 10 to 14 day intervals[24].

It has been speculated that foliar sprays of K could have a positive effect on droughted cotton seedlings because of the important role that K plays in the water relations of plants. This theory was tested in a series of pot experiments in the growth room where conditions could be carefully controlled[41]. These studies and associated field tests indicated that foliar-applied K did not improve the drought tolerance of seedlings, i.e., plant water relations were not improved for continued growth during the stress. Therefore, the potential benefit from such applications is not sufficient to warrant their use. Recent research has suggested that applying foliar fertilizers after relief of the drought stress to stimulate recovery and enhance growth may be beneficial (E.M. Holman, unpublished data), and a similar response may occur with K.

Leaf Uptake of Foliar-Applied Potassium

Understanding the absorption and translocation of foliar-applied K in the cotton plant is important in order to be able to predict how rapidly, and in what amounts, the foliar-applied K is taken up by the leaf and how quickly it moves to the developing boll. Using $^{42}KNO_3$ applied to the midrib of cotton by micro pipette, Kafkafi[48] in Israel showed that foliar-applied K moved into the leaf and to the boll within 20 hours. However, no information was provided on the quantity taken up by the leaf or the time intervals for translocation to the boll. Preliminary studies in Arkansas in 1990, using Rubidium to monitor K movement into the leaf, indicated that K first entered the leaf within 6 hours and then in greater quantities between 6-48 hours after application and was translocated to the developing bolls with little delay during the same period (Oosterhuis and Hurren, unpublished data). Further evidence that foliar-applied K is translocated to the boll was provided by Oosterhuis et al.[71], who demonstrated in field studies that foliar-applied KNO_3 increased K concentration, K content of the fibers, and fiber dry weight (**Figure 2**) compared to the untreated check. More detailed information on the time course of K uptake by the leaf and translocation to the developing boll, as well as factors that effect this process, is still needed.

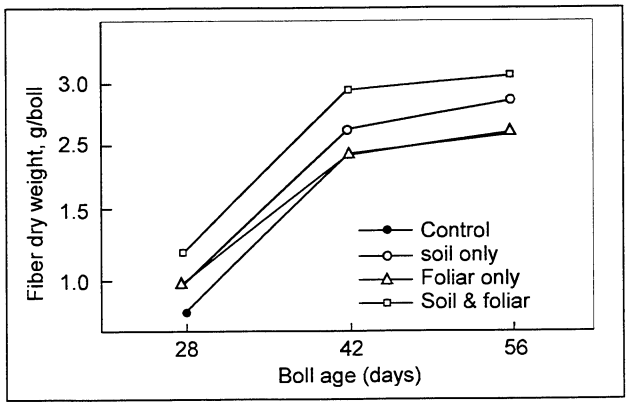

Figure 2. Effect of soil- and foliar-applied KNO_3 on fiber dry weight during boll development (from Oosterhuis et al., 1991b).

Use of Adjuvants with Foliar-Applied Potassium

It has been speculated that the use of adjuvants with the K spray may improve the efficacy of the foliar-applied K fertilizer and thereby provide the potential for decreasing the quantity of K applied

per application. Howard et al.[42] showed that the uptake of K from foliar-applied KNO_3, as reflected in cotton petioles, was increased significantly when surfactants were added to the K solution. However, the final yield of lint was not increased. Similar results have been found in Mississippi (Heitholt, unpublished data) and Arkansas (Oosterhuis, unpublished data). Recent growth room studies by Chang and Oosterhuis[19] showed that lowering the pH of the foliar K solution to between 4 and 6 significantly increased the absorption of K, its subsequent accumulation in the boll, and the seedcotton yield. Additional research is obviously needed to understand the principles involved and, if necessary, to implement these findings into production practices.

Tissue Diagnoses of Potassium

Analysis of soil and plant samples offer a means of determining the K status of a crop. In cotton, tissue tests have become a valuable diagnostic tool for assessing the nutrient status of a crop, for determining fertilizer recommendations during the growing season, and for detecting potential K deficiency[5]. The petiole is generally considered more indicative of plant K status than the leaf blade, partly because of the more rapid decline in K concentration in the petiole, compared to the leaf, during the boll development period[4, 43] (**Figure 3a**). Hsu et al.[43] reported that, although the rates of K decline in both leaf blade and petiole were dissimilar, each was a function of maturity and not a function of K fertilization rate. Although the K concentration of the uppermost mature main-stem leaf petiole is considered to provide a reliable indication of plant K status at the time of sampling[43, 52], Weir and Roberts[91] cautioned that the result may not always provide adequate warning of impending K deficiency. Bednarz and Oosterhuis[9] suggested petioles sampled lower in the canopy than the currently recommended fourth node from the terminal may be more indicative of a pending K deficiency. There is also some concern about the validity of petiole analysis and the resulting recommendations from samples taken later than three weeks after the start of the flowering and boll development period[55].

Obtaining a representative sample from a cotton field is essential for reliable estimates of crop K status. This necessitates a sufficient number of petioles per sample for analysis (usually about 10 to 20), an adequate number of samples to account for field variability, and consistency in sample selection, i.e., the same time of day and position on the plant. A gradient exists of increasing K concentration in the petioles of leaves at progressive main-stem nodes down the plant (Bednarz, unpublished data) which is presumably related to the age of the leaf[43] and its physiological activity. Samples taken after two days of overcast weather may exhibit a 10 percent decrease in K concentration from the previous day (Oosterhuis, unpublished data).

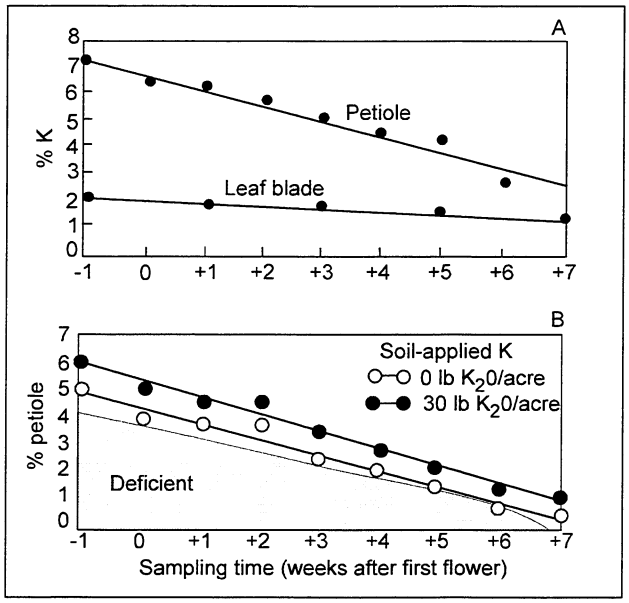

Figure 3. Cotton petiole K status from one week before first flower until boll maturation showing (A) a comparison between the decline in petiole K and leaf blade K, and (B) suggested critical cotton petiole K levels; Marianna, Arkansas (from Baker et al., 1992).

There is still some question about the appropriate critical or threshold levels for K concentration in the leaf or petiole, as these values may be appreciably altered by the environment, plant genetics, and sampling procedure. The sufficiency levels of K in petioles for a cotton crop are generally in the range of 4.0 percent at first flower, 3.0 percent during peak flower, 2.0 percent by first open boll, and 1.0 percent prior to harvest[7, 83, 84]. The critical tissue level for K in cotton leaf blades in mid-to-late season is between 0.9 and 1.2 percent[4] and may be as low as 0.6 to 0.9 percent for significant decreases in leaf photosynthesis[9].

Foliar applications of KNO_3 to cotton on soils moderate to low in K have been reported to significantly increase K concentration of petioles compared to control plants not receiving foliar-applied K[70]. The changes in plant K status from a single foliar application of K may not always be large enough to be detected by petiole analysis[93]. However, the early detection of K deficiency by in-season monitoring using weekly petiole analysis will allow some limited response by producers if yield potentials warrant additional K[4].

Recent developments with small portable selective ion meters using expressed leaf or petiole sap may provide another practical means of obtaining estimates of plant K status[60]. However, it is difficult to obtain a representative sample with such instruments (W.H. Baker, personal communication). Hodges and Baker[40] reported difficulties in expressing sufficient sap for the test, even under non water-stressed conditions. Additional research is required before these instruments can be used with any accuracy or dependability.

Luxury consumption of K , defined as uptake and accumulation of K above levels needed for normal growth, can occur in cotton[47], and this could possibly confuse tissue diagnostic recommendations[9]. Bennett et al.[10] showed that cotton plants continue to accumulate K at rates above that needed to produce maximum yields, with the highest K content occurring in older leaves and petioles. However, Kafkafi[47] suggested that luxury consumption of K can be beneficial for high yields and a cheap source of insurance against possible K deficiency problems.

Optimum Rate and Timing of Applying Foliar Potassium

The timing of foliar sprays, particularly in regard to the growth stage, can be critical in relation to the optimum efficacy of the foliar treatment, and more attention should be given to it[1]. It has been suggested that the recommended growth stages in cotton for foliar-applied K are at the pinhead and first flower stages and at peak boll development[20]. More recent evidence suggests that the optimum timing was during the boll development period starting soon after flowering and continuing at 7 day intervals for 5 weeks (Oosterhuis, unpublished data). Weir and Roberts[93] showed that the optimum stage of response to foliar application of KNO_3 was three weeks after first flower.

In a field study on a Loring-Calloway silt loam soil in Arkansas, six weekly applications of 2.8 kg/ha, 5.6 kg/ha or 11.2 kg/ha of KNO_3 were applied starting at first flower (Oosterhuis, unpublished data). The soil had a low preplant soil K level of 188 kg K/ha. Results suggested that weekly applications of 2.8 kg KNO_3/ha had no significant effect on lint yield compared to the untreated control. Whereas weekly applications at the 5.6 kg KNO_3/ha rate caused a 121 kg/ha (12.5 percent) yield increase, and weekly applications of 11.2 kg KNO_3/ha resulted in a 298 kg/ha (30 percent) increase in yield. Applying KNO_3 according to the K use uptake curve of the developing boll load (e.g. 2.8, 2.8 5.6 11.2, 11.2, 5.6, 5.6, and 5.6 kg KNO_3/ha at weekly intervals from flowering to 8 weeks after flowering, respectively) resulted in the largest increase in yield of 398 kg/ha (41 percent).

No visible injury of cotton leaves was observed at foliar application rates of up to 22.4 kg KNO_3/ha [70] in 94 L water/ha. However, solubility in cold water may be a problem at rates near 10 kg KNO_3/ha.

Sources of Potassium for Foliar Fertilization

A 3-year field comparison of the major K fertilizers was conducted in Arkansas on a Loring-Calloway silt loam[57] with a moderate soil K status (Mehlich 3 extractant) of 200 to 237 kg K/ha in the surface 15 cm and 176 to 197 kg K/ha at 16 to 30 cm of the soil. Salts of K used included nitrate, sulfate, thiosulfate, chloride and carbonate, applied at a rate equivalent to 11.2 kg KNO_3/ha in 93 liters of solution/ha. For the control and each treatment containing a source other than KNO_3, 1.5 kg N/ha as urea was applied to equal the N rate supplied by the KNO_3 treatment.

Results showed a trend for KNO_3 to increase yield the most, followed closely by potassium thiosulfate and potassium sulfate (K_2SO_4; **Table 3**). Potassium chloride (KCl) and potassium carbonate (K_2CO_3) had no effect on yield, and K_2CO_3 significantly decreased yield in 1992[57]. The detrimental effects of K_2CO_3 on yield and the lack of effect on yield of KCl were related to physiological effects on leaf photosynthesis and cell membrane integrity (Oosterhuis, unpublished data).

Only very minor and non-significant visual symptoms of foliar burn were observed following foliar application of any of the K fertilizers. Symptoms consisted of a few small spots on the leaf, particularly with K_2CO_3. A recent study by Chang and Oosterhuis[19] confirmed these findings and showed clear differences among foliar applied K sources in affect on leaf burn, leaf expansion, K absorption, and yield.

Recently there has been some interest across the Cotton Belt in using KCl because it is a cheaper source of K. Pettiet[74] reported that KCl dissolved more easily than KNO_3 and was more easily absorbed by the leaf; however, the effects on yield were not recorded. There have been six field tests across the Cotton Belt in recent years comparing KNO_3 and KCl and in all but one test, KCl had

Table 3. Effects of foliar applications of five K sources on cotton yield and boll weight (from Miley and Oosterhuis, 1994).

	Yield			Boll weight		
Potassium Source [1]	1991	1992	1993	1991	1992	1993
	kg lint/ha			g/boll		
K sulfate	1,186 a[2]	1,146 bc	588 a	4.64 a	4.20 ab	3.85 ab
K nitrate	1,160 a	1,257 a	625 a	4.81 a	4.33 a	3.84 ab
K thiosulfate	1,154 a	1,185 b	552 a	5.05 a	4.28 ab	3.84 ab
K chloride	1,107 a	1,150 bc	569 a	4.84 a	4.27 ab	3.91 a
Check	1,091 a	1,164 b	585 a	4.82 a	4.24 ab	3.84 ab
K carbonate	1,066 a	1,095 c	553 a	4.82 a	4.12 b	3.64 b

[1]Treatments were applied at 2, 4, 6, and 8 weeks after the start of flowering in 1991 and at 2, 4, 6, 7, and 8 weeks after the start of flower in 1992.

[2]Numbers within columns followed by the same letter are not significantly different (P=0.05).

either no effect on yield or decreased yield (W.R. Thompson, personal communication). A salt index has been used as a measure of the effect of a fertilizer on the osmotic potential of the soil solution[76] and may give some insight into the possible effect that a fertilizer could have on leaf tissue. The salt index is defined as the ratio of the increase in osmotic potential produced by a fertilizer material as compared to that produced by an equal weight of sodium nitrate based on the relative value of 100. The salt index for KNO_3 is 73.6 compared to 116.2 for KCl, further indicating that KCl in large concentrations could possibly have a negative influence on leaf tissue. In support of this it has been shown that foliar-applied KCl adversely affected membrane integrity of leaf discs compared to the untreated control and the foliar KNO_3 treatment (Oosterhuis, unpublished data).

Genotypic Differences in Response to Foliar-Applied Potassium

Most of the research on genotypic responses to K fertilization has been conducted using soil-applied K, with only one reference to foliar-applied K. Significant genotypic differences in response to soil-applied K have been reported. Cassman et al.[18] demonstrated significant genotypic differences between two Acala cultivars in K requirement and response to late-season K deficiency. These authors related this to differences in root growth after peak flowering and root-growth response to bulk density. Furthermore, on soils with K problems, it has been suggested that cultivars tolerant to K deficiency will have reduced symptoms and will produce 12 to 40 percent higher yields than more-sensitive cultivars[3, 92]. It is curious that Weir et al.[92] reported no differences in petiole K between two Acala cultivars in response to soil-applied K.

In contrast to the reports on genotypic differences in Acala cottons, Mullins and Burmester[61] reported a lack of difference among cultivars in their total nutrient uptake. Pettigrew et al.[75] suggested that the same genotypes should be used under both K deficient and K sufficient conditions.

A study of genotypic responses to foliar-applied K[46] indicated that there were no significant differences among cotton cultivars in response to foliar applied KNO_3. In their studies, foliar-applied K increased the K concentration in all plant parts of all cultivars studied although this was not always reflected in increased yield.

Effect of Foliar-Applied Potassium on Disease

Soil-applied K has been reported to reduce the incidence of Verticillium wilt (*Verticillium dahliae* Kleb.)[30], although the physiological reasons for this are not clear. In California, K deficiency symptoms have often been associated with the occurrence of Verticillium wilt[56], and the symptoms may be limited to cotton fields infested with Verticillium wilt[92]. Also, soil fumigation to reduce the incidence of Verticillium wilt eliminated the foliar symptoms of K deficiency and Verticillium wilt[92]. Furthermore, varieties more tolerant of Verticillium wilt are often more tolerant of late-season K deficiency[3, 92]. Verticillium wilt blocks the vascular system of cotton, preventing movement of K to the developing boll load or upper canopy leaves[3], causing the K shortage as manifested in K deficiency symptoms. Minton[59] and Ebelhar[58] reported that in soils infested with Verticillium wilt and root knot nematode (*Meloidogyne incognita*), soil-applied K could be used to reduce the Verticillium wilt-K deficiency symptoms.

Recent research in Arkansas has indicated that foliar-applications of K reduced the incidence ($P=0.10$) of Verticillium wilt from 36.1 percent in the untreated control (no foliar applications of KNO_3) plots to 27.8 percent in the plots treated with foliar-applied KNO_3 during flowering.

In what may be a similar relationship, the incidence of Alternaria leaf spot was significantly reduced when treated with a fungicide and K[37]. This was also demonstrated in Tennessee, where an application of a foliar fungicide foliarly applied at 1.15 or 4.64 L/ha with KNO_3 at 11.9 kg K_2O/ha, significantly reduced the incidence of Alternaria leaf spot (M.A. Newman, personal communication). Obviously, additional research is needed to explain the relationship between the occurrence of disease, such as Verticillium wilt, and the appearance of K deficiency.

Factors Affecting Absorption of Foliar-Applied Potassium

Compatibility of potassium and insecticide mixtures.

Foliar applications of K are routinely added to foliar applications of pesticides. Questions have arisen about the compatibility of KNO_3 and insecticides[51]. These authors reported decreased efficacy and a 35 to 75 percent reduction in the amount of pyrethroid insecticide recovered when tank mixed with urea (23 percent N) solution. This was caused by separation of the pyrethroid out of suspension in the urea solution mixture and not due to chemical degradation of the pyrethroid (Graves, personal communication). It has been suggested that similar problems may occur with tank mixes of KNO_3 and insecticides. Recent studies by Baker et al.[5] reported that the pyrethroid insecticide Cymbush 3EC mixed with KNO_3 remained well dispersed with mild agitation and would not be expected to pose a physical compatibility problem. They did report some problems with urea-insecticide mixtures which could be lessened by first mixing the insecticide with water to provide a stable emulsion dispersion. Baker et al.[5] suggest that because of the unique behavior of specific emulsified insecticides, each insecticide/fertilizer combination should be evaluated separately for compatibility to obtain

a high level of assurance of the mixture stability before use.

Urea and KNO_3 mixtures.

Foliar application of urea to cotton is a common practice in the U.S. Cotton Belt and questions have arisen concerning the mixing of urea and KNO_3 in foliar applications. In field studies in Arkansas, rates of up to 11.2 kg N/ha as urea and 11.2 kg KNO_3/ha were tank mixed and applied with a CO_2 backpack sprayer to the cotton in 93 liters of water. It was concluded that mixing KNO_3 with urea did not have any detrimental effect on yield (Oosterhuis and McConnell, unpublished data).

Water deficit stress.

The variable response to foliar K fertilization may also be related to the water status of the plant. Water deficit has been shown to significantly decrease the amount of foliar-applied ^{15}N absorbed by the cotton leaf[98]. Periods of water deficit stress can increase the thickness of the cotton leaf cuticle by up to 30 percent and also alter the composition of the cuticle to more long chain hydrophobic waxes[66] thereby reducing penetration of foliar-applied chemicals. Furthermore, the thickness of the cotton leaf cuticle also increases during ontogeny, while the uptake of foliar-applied ^{15}N decreased concomitantly[13]. The effect of water deficit and cuticle thickness on the absorption of foliar-applied K by cotton has not been documented.

Miscellaneous factors affecting the efficacy of foliar potassium sprays.

The absorption of K by leaves from foliar sprays can be affected by the choice of the salt, the concentration, additives such as adjuvants or insecticides, dew or surface moisture on the leaf, the site of application, leaf age, plant status, and root temperature. Experience in Arkansas has shown that excessively high midday temperatures and low humidity tend to decrease the amount of nutrient absorption by the leaf (Oosterhuis, unpublished data). Dew can enhance the uptake of residue from the foliar fertilizers remaining on the leaf after excessive evaporation[98].

Yield Enhancement Using Foliar-Applied KNO_3 and Plant Growth Regulators

Earlier work at the University of Arkansas has shown a consistent and significant increase in cotton yields from the plant growth regulator PGR-IV[69, 89]. This growth regulator is purported to increase boll retention. In theory, this should further increase the need for additional K. In a field test in Arkansas[64], the following treatments were compared: (a) an untreated control, (b) KNO_3 foliar applied at 11.2 kg/ha at 2, 4, 6, and 8 weeks after first flower (c) KNO_3 at 292 ml/ha at pinhead square and first flower, and (d) PGR-IV followed by KNO_3 (treatments b and c). Results showed that foliar KNO_3 increased seedcotton yields significantly by 61 kg/ha, PGR-IV increased yields by 107 kg/ha, whereas the PGR-IV plus KNO_3 treatment increased yields by 256 kg/ha. A similar trend was recorded with the plant growth regulators, Pix™ and Cytokin™ [67].

Advantages and Disadvantages of Foliar Fertilization with Potassium

The advantages of using foliar feeding with K include low cost, a quick plant response (increased tissue K concentration and fewer new deficiency symptoms), use of only a small quantity of the nutrient, quick grower response to plant conditions, compensation for the lack of soil fixation of K, independence of root uptake problems, increased yields, and improved fiber quality.

On the other hand, the disadvantages are that only a limited amount of nutrient can be applied in the case of severe deficiencies, and the cost of multiple applications can be prohibitive unless incorporated with other foliar applications such as pesticides. Other disadvantages when using high concentrations of K include the possibility of foliar burn, compatibility problems with certain pesticides, and low solubility of certain K salts, especially in cold water. Another restraint is the lack of a full understanding of this technology, specifically the optimum rate and timing, tissue threshold levels to predict the need for foliar-applied K, the physiological mechanism of absorption, and the effect of plant condition and environmental factors on absorption.

Suggestions for Optimum Foliar Fertilization with Potassium

The requirement for foliar-applied K varies greatly with geographical area and even within a single field, and it is difficult to provide a standard recommendation for the practice. Furthermore, the explanation for the cause(s) of the K deficiency syndrome is still not clear. However, research results and practical experience in commercial fields have indicated the following general principles for foliar fertilization with K.

Foliar application of K during boll development may be beneficial when the soil K level is inadequate, from K fixation, low soil test K status, or poor root growth, and when petiole analysis indicates a pending shortage of K. The petiole threshold level of K will decrease from about 4.0 percent at first flower to about 2.0 percent near open boll. Three to four foliar applications of K should be made during the first five weeks of boll development at 7 to 10 day intervals starting at the commencement of flowering. A minimum rate of approximately 4.5 kg/ha of K should be used at each application. The recommended source of K for foliar fertilization is KNO_3, although K_2SO_4 or $K_2S_2O_4$ appear to work almost as well. Attention should be given to possible solubility problems in cold water. The use of an adjuvant with the foliar spray will increase leaf K uptake but may not necessarily result in increased yields, although it may permit the use of a

lower rate of K per application. Further insight into the practical applications of foliar fertilization of cotton with K are give by Roberts et al.[79].

Conclusions

Potassium deficiency has occurred widely across the U.S. Cotton Belt in recent years. The occurrence of these outbreaks of K deficiency has been somewhat unpredictable and the explanations not clear. The K-deficiency syndrome appears to be a complex anomaly related to low soil K status, K fixation in the soil, a greater demand for K by modern cultivars, less storage of K prior to flowering by modern cultivars, the inability of the root system to supply the needed K during boll development, and possible relationships with diseases such as Verticillium wilt. Interest has focused on the possibility of foliar feeding with K to supplement traditional soil application methods. Foliar applications of K offer the opportunity of correcting these deficiencies quickly and efficiently, especially late in the season when soil application of K may not be effective. Research during the last five years has shown that foliar-applications of KNO_3 can alleviate K deficiency and significantly increase yield and fiber quality. However, results from across the Cotton Belt have been variable and unpredictable, and additional research is needed to fully explain this phenomenon. There is sufficient evidence that foliar application of KNO_3 appears to be a useful production practice for supplementing preplant soil applications of K fertilizer, especially when K deficiency symptoms occur and soil and petiole tests show a low K status.

Synopsis of Future Research Imperatives

Additional work is still required to elucidate the chain of events occurring during the onset of K deficiency. This is certainly the case in cotton, where, by the time a deficiency is detected, it is often too late to remedy the situation, and reduced yields and fiber quality result. An improved understanding of K deficiency in cotton could ultimately lead to more reliable indicators of a pending deficiency for more timely management inputs for optimum yield and fiber quality. A better knowledge of the physiology of K deficiency should also help to explain the inconsistent response to foliar-applied K. Lastly, the effect of water deficit on K partitioning and response to soil and foliar applications of K needs to be quantified.

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Chapter 12:
Potassium Deficiency and Carbohydrate Metabolism in Citrus

R. Lavon
Agricultural Research Organization, Institute of Horticulture, The Volcani Center, Bet Dagan, 50250 Israel
E.E. Goldschmidt
The Hebrew University of Jerusalem, The Kennedy-Leigh Centre for Horticultural Research, Faculty of Agriculture Rehovot, 76100 Israel

Introduction

The mineral nutrition of citrus trees (*Citrus* sp.) has been studied extensively. Considerable attention has been paid to the symptoms and consequences of potassium (K) deficiency[25, 52]. It has been long ago recognized that K is one of the most important mineral constituents of citrus fruit and that large amounts are removed annually by the crop[18, 90].

The impairment of a certain plant system under mineral deficiency could be indicative as to the specific role of the missing element. Unlike some other mineral elements, no single biochemical role could be assigned to K in plant nutrition. However, its central physiological role in stomatal opening is well established[42, 94].

Huber[43] was one of the first to draw attention to the interference of K deficiency with several aspects of carbohydrate metabolism. Following this line, Lavon et al.[57] were able to demonstrate a role for K in the starch-soluble sugar conversions of citrus leaves. In the present review article we discuss the available evidence concerning links between K nutrition and carbohydrate metabolism in citrus.

Citrus Requirements for Potassium

Potassium is the most abundant mineral in the citrus fruit (**Table 1**). It occurs mainly in the form of soluble K salts of the organic acids in the juice[87]. Citrus fruits serve as a strong sink for K since more K is accumulated by the fruit than any other mineral element[17, 90]. Severe K deficiency will reduce fruit production[19, 48, 51].

Table 1. Amounts of various elements removed from the soil by the orange fruit (fresh weight).

Element	A†	B‡
	---------- lb/ton ----------	
K	4.50	3.90
N	2.35	2.78
Ca	2.10	1.00
P	0.55	0.32
Mg	0.40	0.42
S	0.25	—
Fe	0.0055	—
B	0.0050	0.0043
Mn	0.0015	0.0027
Zn	0.0015	0.0043
Cu	0.0010	0.0015
Al	0.0032	0.0045

† Adapted from Chapman and Kelley[18].
‡ Adapted from Smith and Reuther[90].

Potassium is phloem mobile[66] and seems to be translocated from leaves to satisfy the needs of the fruit. Indeed, leaves from non-fruiting shoots of the Owari Satsuma mandarin contained higher concentrations of N, P, and K than leaves of fruiting shoots[20]. Within the fruit, juice is a strong sink for K and its level is nearly eight times higher than the levels of both Ca and Mg[91]. Its concentration in the juice was affected to a lesser extent than in the leaves and flavedo under potassium-deficient conditions (**Table 2**).

Table 2. Concentration of K in the leaves, peel (flavedo) and juice of Shamouti orange. Lavon, 1994 (unpublished data).

Nutritional treatment	K-leaves	K-flavedo	K-juice
	------ % dry wt. ------		mg/100 ml
NK	1.09	1.38	129.6
NP	0.85	0.90	147.9

Potassium and Crop Load

The concentration of K in the leaves normally declines throughout the season, and fruit load accentuates the decrease of leaf K during the period of fruit growth and maturation. This can be clearly seen in an alternate bearing variety such as the Kinnow mandarin (**Figure 1**).

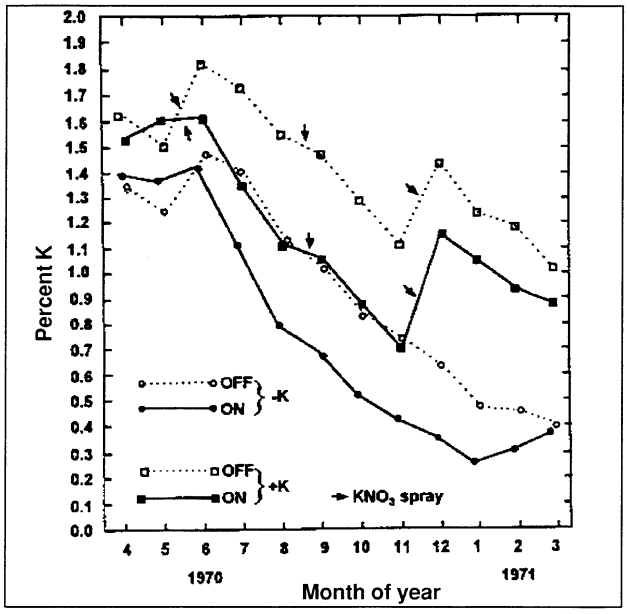

Figure 1. Relation of leaf potassium to fruit load, time, and foliar sprays of KNO_3 in alternate bearing Kinnow mandarin trees. Modified from Jones et al.[47].

Alternate bearing is a typical phenomenon of many fruit trees[70]. The intensity of alternate bearing in citrus varies among cultivars and is ubiquitous to mandarin varieties such as Wilking, Kinnow and Murcott in which trees often collapse after a heavy fruit load (an "on" year)[80, 92]. Calculation of the total tree mineral balance of a Wilking mandarin tree showed that the amounts of K present in the fruit during an "on" year represented 57.8 percent of the total K in the tree whereas fruit N and P were 32.2 percent and 43.7 percent of the tree total, respectively[36]. In all other organs, the K was lower during an "on" year than during an "off" year (**Table 3**). Nevertheless, total K per tree was much higher in the "on" tree, indicating increased uptake of K from the soil during an "on" year (**Table 3**). Similarly to citrus, K constitutes about 70 percent of the mineral cations in ripe grapes[37].

Table 3. Total amount of potassium percent in different organs of "on" and "off" Wilking mandarin trees. Golomb and Goldschmidt[36].

	Status of productivity	
Tree organ	"On"	"Off"
	g K/tree	
Fruits	255	—
Leaves	12	71
Twigs	11	27
Tree skeleton	132	141
Roots	28	61
Minor roots	3	5
Total per tree	441	305

Yield reduction in Murcott mandarin, following an "on" year, was attributed to depletion of N, P, and particularly K from the leaves, and application of K ameliorated the collapse of the trees[92]. The changes in K levels in "on" year trees of Kinnow and Murcott were associated with a decrease in the starch content of the roots[46, 47, 89]. Carbohydrate reserves of Kinnow were completely depleted, and there was no starch in tree organs after an "on" year[46]. Fruit load of these trees reduced the concentration of P and K in the leaves but not to a deficiency level. Addition of K had no effect on starch accumulation and alternate bearing[46], but caused a transient increase in leaf K level (**Figure 1**). Some reports claimed, however, that addition of KNO_3 as a foliar spray minimized alternate bearing of mandarin Balady trees[24]. Carbohydrate depletion following an "on" year was also reported by Goldschmidt and Golomb[34] for Wilking mandarin trees. Large sized starch granules which were abundant in leaves, bark and pith of twigs and roots from "off" Murcott trees were absent in "on" year trees[65]. The soluble sugar pool was also reduced under heavy crop load, but to a lesser extent than starch[34, 72].

Satsuma mandarin trees with high fruit/leaf ratio absorbed more P, K, and Mg while the absorption of water, nitrates and Ca declined[41]. The correlation between K and starch found in alternate bearing trees can be clearly seen also in rough lemon leaves deficient of K, which contained lower starch levels. The starch granules were smaller than those of control leaves[57].

Strong antagonistic effects exist among N, P, and K[88]. Increasing the N levels decreased the P and K levels as well as carbohydrate concentration and C:N ratio of Balady lime trees. When K levels were increased, leaf area and C:N ratio were also increased[64].

The chain of events described for citrus is quite similar to that of deciduous trees. Leaves of fruiting apple trees were lower in K and higher in Mg, N, P, and Ca than leaves from non-fruiting trees. In fruiting trees, glucose and sucrose accumulated while starch content was reduced[96]. Similarly, one-year old shoots, limbs, bark, and roots of fruit-bearing French prune trees had lower starch content than those of non-bearing trees, but no differences in the soluble carbohydrate content, nor in the ratio of sorbitol + sucrose/glucose + fructose were observed. Heavy cropping of this cultivar induced K deficiency which stemmed from the strong demand for K by the fruit and from lack of carbohydrate translocation to the roots[38].

Fruit Set and Abscission

In citrus the number of harvested fruits is usually less than 0.1 percent of the flowers formed because a large number of the reproductive organs abscise prior to fruit harvest[69]. Competition for either carbohydrates or nutrients is among the factors determining abscission[28, 32]. The carbohydrate source-sink relations and their regulatory function in citrus have been critically reviewed by Goldschmidt and Koch[35].

The relationship among fruit set, mineral nutrients, and carbohydrate levels were studied by Sanz et al.[84]. A decrease in the concentration of P (46 percent), K (31 percent), and N (6.7 percent) was found in old leaves of Washington navel orange trees during spring flush and flower opening. Nitrogen and K were recovered one month after full bloom but a transient decrease in the level of K occurred again during June drop. During the same period a decrease of P and K levels was evident also in young leaves[84]. The abscised fruitlets had lower levels of N, P and K than persisting fruitlets which coincided with higher invertase activity and accumulation of reducing sugars in the abscising fruitlets[81]. Similarly in apples, abscised fruits had higher levels of reducing sugars and sucrose and lower concentrations of K, acid hydrolysable polysaccharides, and protein[1]. Lekvinadze[60] studied the effect of different K_2O rates applied to Satsuma mandarin trees (50, 100 or 200 g/tree) in addition to NP fertilizers and reported that all K application rates stimulated carbohydrate accumulation. Addition of K_2O to NP fertilizers at a rate of 100 to 200 g/tree increased the leaf carbohydrate content of mandarin leaves during winter and improved trees' frost resistance[49].

Fruit Size and Yield

Low K levels reduce yields by producing small size fruit and increasing fruit split and preharvest fruit drop[52]. Foliar application of K together with 2,4-dichloro-phenoxyacetic acid significantly increased fruit size by as much as 8 to 20 percent for Shamouti and 8 to 25 percent for Valencia oranges[29]. The same spray combination given to Nova tangerines reduced fruit splitting, increased fruit size and improved yield (**Table 4**).

Table 4. Foliar spray treatments and their effect on leaf K, fruit weight, percent of split fruit and yield of Nova tangerines. Lavon, 1994 (unpublished results).

Spray treatment†	Leaf K, % dry wt.	Fruit wt., g	Split fruit, %	Yield, kg/tree
Control	0.50 d‡	117 c	27.5 a	47.4 c
KNO_3 (1 spray)	0.50 d	123 abc	18.0 b	54.7 bc
2,4-D (1 spray)	0.69 c	123 abc	15.0 bc	65.4 ab
KNO_3 + 2,4-D (2 sprays)	0.79 b	130 ab	15.0 bc	64.2 ab
KNO_3 + 2,4-D (3 sprays)	1.00 a	134 a	11.0 c	68.8 a

† The concentrations of KNO_3 and 2,4-D were 5% and 20 ppm, respectively, in all treatments.
‡ Mean separation within columns by Duncan's multiple range test at P=0.05.

A continuous supply of K through the irrigation system increased fruit size and yield of Shamouti orange trees[55]. Small fruits and low yields were obtained when K was deficient (**Table 5**). These results agree with previous findings that increased K levels increase fruit size, rind thickness, and decrease creasing and splitting of the peel[4, 26, 53, 58, 79, 88]. Different N/K ratios are required for obtaining maximum yield or optimum fruit size[23].

Sugar:Acid Ratio

Citrus trees receiving greater amounts of K produce fruits with higher juice acidity (lower pH)[27, 55, 87]. High concentrations of K in the Murrumbidgee irrigation areas of Australia caused poor fruit quality with high acidity, low juice content, and thick peel. High P levels increased juice content and were correlated with low acidity[58, 71]. Potassium nutrition of citrus trees has a beneficial effect on the ascorbic acid content of citrus fruits[87]. The total soluble solids (TSS):acid ratio is consistently reduced by increased K level[52, 55]. Foliar application of KCl to low K lemon trees increased fruit yield and improved fruit quality by increasing TSS, ascorbic acid and total sugar contents of the fruit[78]. Calamondin fruits from plants grown in K-deficient nutrient solutions had distinctly lower amounts of TSS and acid and yet their maturity ratio (TSS/acid) was higher than that of fruits from plants grown in full nutrient solutions[54] (**Table 6**).

Fertilization pot experiments of Satsuma mandarin trees receiving five levels of K showed that when K was deficient the concentration of soluble sugar in the fruit increased. When K level was increased, the yield, fruit size and citric acid content of the juice were increased[39]. Marsh seedless grapefruit showed high positive correlation coefficients among fruit circumference, acidity, TSS, and K content. Acidity tended to decrease, however, in large-sized fruits[2].

A low level of available K until harvest increased the concentration of mono and disaccharides in apple. During the second cropping year, high levels of available K in the summer reduced sugar and increased acid levels in the fruit[63].

Unlike citrus fruit, high levels of K in grapes decreased fruit acidity and caused high pH wine problems[14]. A highly significant positive correlation was found between malic acid and K levels in grape juice[10]. The higher titratable acidity may be accounted for by the higher malate content. The higher pH was probably due to the greater proportion of malic acid which is a weaker acid[37].

Table 5. Fruit weight, yield and fruit quality of Shamouti orange as related to K concentration in the leaves. Mean values of a 9-year fertilization experiment. Lavon et al.[55].

Fertilization treatment	Leaf-K, % dry wt.	Fruit wt., g	Yield ton/ha	,TSS†, %	Acid, %	TSS/acid, ratio
N,P,K	0.84 a‡	226 a	49 a	12 a	1.41 a	8.6 c
N,P	0.57 b	194 b	44 ab	12 a	1.25 c	9.8 a
N	0.60 b	191 b	42 b	12 a	1.35 b	9.2 b

†Total soluble solids.
‡ Mean separation within columns by Duncan's multiple range test at P=0.05.

Table 6. Effect of K, Mg and Ca deficiencies on leaf K, fruit weight and internal juice quality of Calamondin fruit. Lavon[54].

Nutritional treatment	Leaf K, % dry wt.	Fruit wt., g	TSS, %	Acid, %	TSS/acid, ratio
Control	0.50 a†	13.2a	15.6 b	7.6 b	2.0 b
K-deficiency	0.14 b	3.9 b	9.1 c	3.9 c	2.4 a
Mg-deficiency	0.58 a	14.5 a	11.7 c	7.2 b	1.6 c
Ca-deficiency	0.58 a	4.6 b	18.7 a	10.2 a	1.8 bc

† Mean separation within columns by Duncan's multiple range test at P=0.05.

Starch Formation/Degradation and Hexose Accumulation

Accumulation of soluble sugars and the decreased levels of starch in low K plants were reported in the early thirties by Janssen and Bartholomew[45] in cowpea plants. Huber[43] found lower starch and higher sucrose concentrations in K deficient soybean leaves at the end of the photoperiod, compared with K sufficient leaves. Sucrose concentrations can either be reduced or elevated in K deficient leaves[43, 44]. Higher concentrations of sucrose and reducing sugars were found in primary leaves of bean plants deficient of K and Mg as compared with control and P deficient leaves[15].

In rough lemon plants grown in K deficient nutrient solutions sucrose and soluble sugars accumulated while starch levels decreased[54]. The starch:soluble sugar ratio was significantly lower in K deficient leaves than in control leaves (**Table 7**). Deprivation of K from one-year-old sour orange and rough lemon seedlings elevated their stem carbohydrate content and reduced their dry weight[67]. Similar trends were observed in other plants. The contents of reducing and non-reducing sugars in K deficient tea shoots were reduced following K application[21].

Table 7. Concentrations of starch, sucrose, soluble sugar, and the starch:sugar ratio of rough lemon leaves deficient in K, Mg and Ca. Determinations were made on fully expanded leaves grown for 3 months in the nutrient solutions. Lavon et al.[57].

Nutritional treatment	Starch	Soluble sugar	Starch: soluble sugar ratio
	mg/g leaf fresh wt.		
Control	37.2 ab†	25.0 b	1.4 ab
K deficiency	16.0 b	38.3 a	0.4 b
Mg deficiency	64.4 a	30.6 ab	2.2 a
Ca deficiency	65.6 a	31.0 ab	2.2 a

† Mean separation within columns by Duncan's multiple range test at P=0.05.

Shoots of "low-K" Valencia orange trees had significantly lower starch levels than of "high-K" trees[12]. It is noteworthy that low starch and accumulation of reducing sugars may be observed in citrus under water stress[98], or chilling injury[73], as a consequence of increased invertase activity induced by low temperatures[77].

The mechanism of sucrose accumulation under K deficient conditions is not clear, since the activity of sucrose phosphate synthase (SPS), a key enzyme in sucrose formation, was decreased. It may perhaps be explained by lower sucrose export rates from the leaf[16, 43]. Osmoregulation of intact bean guard cells was dependent on K transport and sucrose metabolism[94]. Work with isolated guard cells pointed towards photosynthetic C fixation, starch degradation or import of apoplastic sucrose as possible sources for sucrose accumulation[94].

The positive correlation between leaf K and starch may be attributed to the stimulation of starch synthase activity which catalyzes the conversion of ADP-glucose to starch[40]. Applying tissue culture techniques to the study of salt tolerance in citrus, Ben-Hayyim and Kochba[7] demonstrated that cells of salt-sensitive Shamouti orange (L-5) contained higher levels of K than salt tolerant cells (R-10) and had two- to three-fold more starch than the salt tolerant cells when grown in the same control medium[61]. The activity of starch biosynthetic enzymes, ADP glucose pyrophosphorylase and starch synthase were five and three fold higher, respectively, in salt-sensitive than in salt-tolerant cells[61].

However, K did not have a consistent effect on the enzyme activity of ADP glucose-starch synthase of barley grains. The effect of K on barley grain weight was attributed to photosynthesis and translocation rather than to direct effect on grain metabolism[8].

Shoot/root dry weight ratios of rough lemon plants did not change under K deficiency although a decrease in the dry weight of leaves, shoots and roots was evident[54]. In contrast, the shoot/root ratio of bean plants increased under K deficiency and particularly under Mg deficiency, and declined in P deficient plants[15].

Enzyme Systems

Potassium appears to be required for the catalytic activity of several enzyme systems[11, 30, 93]. Studies with pyruvate kinase indicated the importance of K in maintaining the conformation and stability of the protein[31, 99]. Hawker et al.[40] reported that the activity of granule-bound ADP glucose-starch synthase from leaves of sugar beet, bean, and saltbush (*Atriplex nummularia*) was stimulated by K. The activity of ribulose bisphosphate carboxylase (RuBPcase) of K deficient alfalfa leaves increased when the concentration of K in the leaves increased[75]. Thus, accumulation of soluble sugars in K deficient leaves may be correlated with increased activity of acid invertase[43]. It is interesting to note that the decreased starch concentrations and accumulation of soluble sugars in K deficient citrus leaves were correlated with increased amylolytic activity[57].

Pyruvate Kinase

In the glycolytic pathway, K together with Mg plays an important role in the activation of pyruvate kinase, an enzyme which converts phosphoenol pyruvate and ADP to pyruvate and ATP. In K deficient plants hexose accumulation has been related to decreased activity of this enzyme[30]. Activation of pyruvate kinase may be achieved by other monovalent cations, but K is the most effective one[93].

The activity of pyruvate kinase in K deficient citrus leaves was significantly lower from that of the control (**Table 8**)[5, 56]. The reduced activity of

pyruvate kinase in K deficient leaves may be used as a biochemical indicator for detection of K deficiency or cation imbalance in the leaves of horticultural crops[5, 9, 56, 59].

Table 8. Potassium concentration and activity of pyruvate kinase in crude leaf extracts of rough lemon plants deficient of K, Mg and Ca. Activity was measured in the presence of K + Mg in the assay medium. Lavon[54].

Nutritional treatment	K-concentration, mM	Pyruvate kinase activity, nmol pyruvate mg protein/h
Control	23.6	3.15 bc†
K-deficiency	9.1	2.73 c
Mg-deficiency	30.0	3.60 b
Ca-deficiency	19.3	4.61 a

† Mean separation within columns by Duncan's multiple range test at P=0.05.

The correlation between K and pyruvate kinase activity can be demonstrated in other horticultural crops such as apple and olive. Apple fruit with low Ca:(Mg+ K) ratio suffered from high bitter pit incidence and had higher pyruvate kinase activity than fruit with low levels of the disorder[100]. Vegetative buds on non-bearing olive trees had low K content, low pyruvate kinase activity and a high content of reducing sugars, whereas reproductive buds on bearing trees had high K content, high pyruvate kinase activity and a low content of reducing sugars[68].

Amylases

Starch degradation in leaf tissues is associated with complex activities of α-amylase, β-amylase, starch debranching enzyme, starch phosphorylase and α-glucosidase[6]. The physiological role of amylases in vegetative tissues is not well understood. Doehlert and Duke[22] observed that levels of amylolytic activity were higher in stems which were less active photosynthetically than in leaves. Saeed and Duke[82] showed higher levels of β-amylase activity in pea tissues which had lower levels of chlorophyll and starch, and a greatly increased a-amylase activity in senescing tissues. An increase of amylolytic activity in detached and senescenced orange leaves was demonstrated by Arguelles and Guardiola[3]. Separation on native gels indicated that total amylolytic activity contained α-and β-amylases and the differences in band pattern was associated with leaf development[83]. Studies on total amylolytic activity during a yearly cycle indicated that enzyme activities were not correlated with the rate of starch breakdown[83]. In citrus, as in herbaceous plants, total amylase activity was higher in bark of twigs and in roots than in leaves. The activity of β-amylase in leaves was particularly low[65]. Potassium-deficient rough lemon and Calamondin leaves had significantly higher total amylase activity than control leaves (**Table 9; Figure 2**).

Table 9. Concentration of K and total amylase activity (mg reducing sugar g^{-1} fresh wt h^{-1}) in rough lemon and Calamondin leaves. Lavon[54] and Lavon et al.[57].

Nutritional treatment	Rough lemon		Calamondin	
	K	Amylase activity	K	Amylase activity
	% dry wt.		% dry wt.	
Control	1.41 a†	2.1 b	1.07 a	1.9 b
K deficiency	0.51 b	12.0 a	0.36 c	5.3 a
Mg deficiency	1.14 a	3.7 b	1.13 a	3.0 b
Ca deficiency	1.15 a	4.2 b	0.77 b	2.2 b

† Mean separation within columns by Duncan's multiple range test at P=0.05.

Lavon et al.[57] distinguished the activity of α-amylase isozymes from β-amylase isozymes by substituting the starch substrate with β-limit dextrin which cannot be degraded by β-amylase or with amylopectin which produced red stained bands when digested by β-amylase. Two groups of amylase bands were detected on starch gels in crude leaf extracts: a slow electrophoretically mobile (A) and a fast migrating group (B). Both of these groups were more pronounced in K deficient leaves (Figure 3). The activity of group B isozymes was rather weak when the gel was loaded with crude extracts, but 4 to 5 bands appeared clearly after $(NH_4)_2SO_4$ precipitation (**Figure 3**). The stronger activity of K deficient extracts was evident in both isozyme groups with starch as well as with amylopectin, but not when β-limit dextrin was used as a substrate[57]. This suggests that K deficiency induced mainly β-amylase activity.

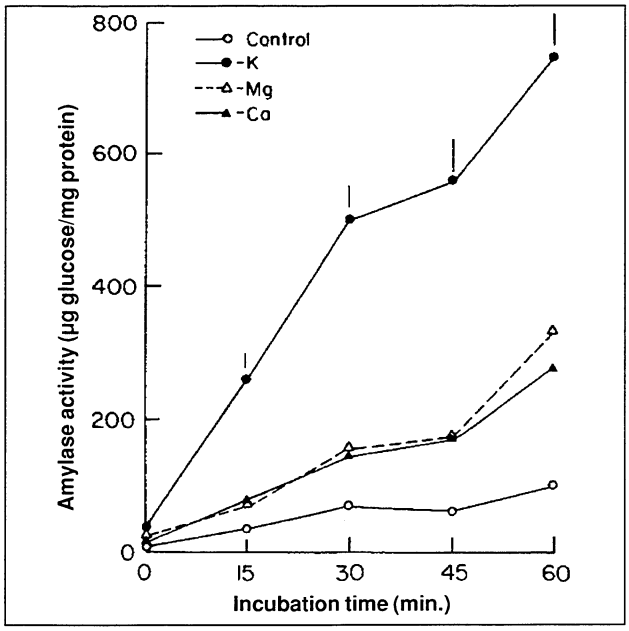

Figure 2. Effect of the incubation time on the release of glucose from starch by amylolytic activity of rough lemon leaf extracts deficient in K, Mg, and Ca. Each bar represents LSD for comparison between means at a 0.05 probablity level. Lavon[54].

Paper chromatography used by Lavon et al.[57] for the identification of the products generated by the amylolytic reaction of citrus leaf extracts *in vitro* revealed glucose and maltose. Accumulation of maltose and an additional faint spot close to the front, tentatively identified as maltotriose, were more pronounced in the K deficient leaves[57]. The liberation of maltose as the major product of amylolytic activity in K deficient leaf extracts further emphasizes the role of β-amylase in the amylolytic activity of K-deficient leaves. The large amounts of malto-triose generated after prolonged incubation with pullulan, indicated the presence of debranching enzyme activity (data not shown). Debranching enzyme activity was more pronounced in roots of Murcott mandarin than in leaves[65].

Figure 3. Amylolytic activity of leaf extracts from control and potassium-deficient rough lemon leaves. Crude extracts were precipitated by 60 percent $(NH_4)_2SO_4$ and separated on non-denaturing PAGE, containing 0.2 percent Lintner soluble starch. Gels were stained with KI-I_2. The amount of protein per lane was 560 µg. Lavon[54].

Invertase

Soluble acid invertase is the primary enzyme involved in sucrose hydrolysis in citrus leaf tissue. Its activity was highest during the period of highest growth rate (60 to 80 percent full leaf expansion) and then dramatically decreased toward and after full expansion[85]. Reducing sugar levels paralleled the acid invertase activity, and their levels rose until full expansion and then declined[85]. In K deficient rough lemon leaves the accumulation of soluble sugars was associated with increased activity of acid invertase (**Table 10**). Similar hexose accumulation and increase in acid invertase activity were found in K deficient soybean leaves[43]

Photosynthesis and Stress Responses

Potassium plays a key role in the stomatal functioning[74]. Impairment of the stomatal function may expose the plant to damage from water stress (and other related stresses) and interferes with photosynthetic activity[76].

'Valencia' orange trees grown in containers low in K exhibited poor stomatal control and under conditions of high evaporative demand suffered from water stress. High levels of K nutrition resulted in better stomatal control, slower rates of water loss, with eventual higher overall CO_2 uptake[12]. Low photosynthetic rates were also evident in field-grown Shamouti orange trees deficient in K (Shahak and Ratner,1996, unpublished data). These trees were also susceptible to high irradiation photodamage[86]. The involvement of K in plant responses to stress deserves further investigation.

Table 10. Acid invertase in rough lemon leaves deficient in K, Mg and Ca. Lavon et al.[57].

Nutritional treatment	Acid invertase activity mg glucose equivalents/g fresh wt/h
Control	1.2 b†
K-deficiency	8.5 a
Mg-deficiency	0.2 a
Ca-deficiency	ND‡

† Mean separation within columns by Duncan's multiple range test at P=0.05.
‡ Nondetectable.

Summary

Carbohydrate availability plays a critical role in most phases of the citrus reproductive cycle[35]. Fruit growth in particular, seems to be dependent upon the supply of carbohydrates[13, 35]. Fruiting-induced depletion of carbohydrates has been shown in the present article to be correlated with heavy consumption of K and the fruit is undoubtedly the strongest sink for K. Small sized fruit have been recognized as one of the consequences of K deficiency in citrus[23, 54, 55, 88]. This further illustrates the pivotal role of K in fruit sizing. Growth of other fleshy fruits, such as grapes, peach and tomato, is also dependent upon the supply of K[14, 33, 62, 97]. Although we cannot identify at the present time a specific, growth limiting, K dependent biochemical reaction, it seems reasonable to assume that K is intimately involved in the provision of carbohydrates for fruit enlargement and maturation. When K is deficient, the starch:soluble sugar balance changes in favor of the latter component. The lower levels of starch in K deficient leaves are correlated with amylolytic activity and accumulation of soluble sugars[57]. The elevated levels of invertase may also account for the accumulation of soluble sugars in K deficient leaves[43, 57]. However, this is not the sole physiological role of K. It has been known for quite some time that K plays a key role in the stomatal apparatus[95]. The recent findings of Bower and Wolstenholme[12] suggest that in citrus, high levels of K are needed for maintenance of a functional stomatal apparatus, thereby ensuring sensitivity to water stress as well as, in the long term, better photosynthetic yields. The reduced photosynthetic rates under K deficiency may, perhaps, be behind the observed induction of starch hydrolysis (**Tables 7 and 9**). The existence of these regulatory relationships requires further, substantial evidence.

Research Imperatives

Further elucidation of the links between K nutrition and citrus carbohydrate metabolism would seem to depend upon identification of specific, K-sensitive biochemical reactions. In particular, the fruit's need for large amounts of K is not well understood. Potassium and carbohydrates may, however, be linked also through nutritional modulation of gene expression, as recently suggested by Koch[50]. In such a case, the specific genes involved must be identified. The existence of signals (metabolic? hormonal?) generated by mineral deficiencies should also be investigated.

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Chapter 13:
Root System Interactions with Potassium Management in Corn

D.L. Allan, G.W. Rehm, and J.L. Oldham

Department of Soil, Water, and Climate, University of Minnesota, St. Paul, MN 55108-6028

Introduction

The subject of this chapter requires that two questions be addressed: the effect of potassium (K) on corn (*Zea mays* L.) root growth; and the effect of the distribution and density of the root system on K status of the corn plant. With respect to the first question, response of root systems to K supply has been the subject of few investigations. In contrast to N and P, there is no localized response of root growth to K application[5, 11]. Instead, a localized supply of K promotes lateral root growth throughout the root system to the same extent as a well-fertilized control[4, 5]. Consistent with these findings, most researchers have seen no effect of K addition or placement on corn root growth in field studies[12, 14]. Stypa and colleagues[12] observed no differences in root growth or distribution in the top 50 cm of a silt loam soil in Guelph at any of three growth stages or two sampling positions. However, the two K treatments in this study were both sufficient, including a recommended and very high rate. Potassium treatments in the study by Yibirin et al.[14] did include low K controls, but root samples were only collected at tasseling, so no early-season effects were measured. Kuchenbuch and Barber[7] found no effect of P or K fertilizer treatment on overall root length measured at 12 weeks after planting. They concluded that growth of corn roots was not sensitive to changes in fertility even though final yield was affected by low K availability.

In a greenhouse pot study, Wolkowski[13] found significant increases in root weight with K addition for two finer textured soils (silty clay loam and silt loam) but not for a loamy sand. Root weight increased at the high-medium and excessive soil K levels about 40 and 55 percent, respectively. However, shoot weight increases were even larger, from 70 to 100 percent. There was no effect of banded K on root growth in this study.

Although corn root growth in the field has not been shown to be sensitive to K supply, there is strong evidence of the importance of such root parameters as density, diameter and distribution in determining crop K uptake[2]. Kuchenbuch and Barber[7] showed that where K levels were low and corn yields correlated with ear-leaf K concentration over a nine year study period, root density in the 0 to 15 cm layer was positively correlated with corn yield ($r=0.80$, $P<0.001$) and ear-leaf K ($r=0.63$, $P<0.05$). Brouder and Cassman[4] showed for cotton (*Gossypium hirsutum* L.) that shoot K accumulation was not related to soil K, but was strongly correlated with mean root surface area density ($r=0.93$, $P<0.001$). Keino et al.[6] found differences in uptake kinetics of two cotton cultivars that appeared to be related to differences in root hair activity and distribution. Corn hybrid differences in P and K uptake were shown by Barber and Mackay[3] to be primarily the result of differences in root growth in the topsoil rather than differences in uptake kinetics, root hair growth, or amounts taken up from the subsoil. For both genotypes on both soils studied, there was a high correlation between root surface area and K uptake ($r=0.93$, $P<0.01$).

Two of our recent studies address questions about effects of K management on corn root response. In one, we measured how root density was affected by plant population density, soil fertility level, and K management. In the other we examined effects of tillage system and K management on the root density and root activity of two corn hybrids. The experimental designs and representative findings are presented below.

Plant Population and Potassium Management

The objective of this research project was to determine optimal strategies for management of fertilizer K for a range of corn plant densities grown on soils with high or low soil tests for K. There has been some increase in corn plant populations used by Minnesota farmers in recent years, and research by Dale Hicks (personal communication) had shown marked increases in grain yields when populations grown with high fertility levels were increased by as much as 70 percent over current averages. However, before recommendations could be made for higher populations, it was necessary to examine plant density, soil fertility, and K management interactions.

Methods. The studies were conducted over three years (1991-1993) on farmer fields in southeast Minnesota. Fields were selected based on soil test levels and soil uniformity. Soils were classified as Timula silt loam (coarse silty, mixed mesic Typic Eutrochrept) or Mt. Carroll silt loam (fine silty, mixed mesic Mollic Hapludalf). For the high fertility sites, two fertilizer treatments (control, 14 kg K/ha in a starter fertilizer) were combined with five planted populations (56,800, 69,200, 81,500, 93,900, and 106,200 plants/ha) in a randomized complete block design with four replications at all sites. High fertility sites had ammonium acetate extractable K concentrations of 245, 248 and 225 mg/kg in the top 15 cm in 1991, 1992 and 1993. At

the low fertility sites, the same plant populations were combined with four fertilizer treatments (control, 37 kg K/ha in a starter fertilizer, 93 kg K/ha broadcast and 186 kg K/ha broadcast). Low fertility sites had ammonium acetate extractable K concentrations of 106, 77 and 92 mg/kg in the top 15 cm in 1991, 1992 and 1993. Adequate P, supplied as 0-20-0, and N, supplied as 46-0-0, were broadcast and incorporated with a field cultivator before planting.

Dry matter and grain yields, K uptake, and root density and distribution data were collected. Root sampling was performed at two growth stages only on selected fertilizer and population treatments (control treatments on both high and low K sites, and 93 and 186 kg/ha broadcast treatments on the low K sites; 56,800 and 81,500 plants/ha). Cores (2.5 cm diameter) were taken at 7.5, 15, and 30 cm from the row, directly opposite a plant and perpendicular to the row. The cores were divided into four depth increments: 0 to 7.5 cm, 7.5 to 15 cm, 15 to 30 cm, and 30 to 60 cm. Four complete sets of cores were taken per plot and composited before washing in a Gillison elutriator (Gillison's Variety Fabrication, Inc., Benzonia, MI). Following washing, root samples were stored in 5 to 10 percent methanol, stained with basic fuschin, handpicked to remove organic debris, and scanned on a Datacopy/WIPS processing system (Model 900, Datacopy Corp., Mt. View, CA). Mean root density (cm/cm^3) was calculated by averaging root densities measured at 3 distances from the row and 4 depths at each position. Mean root length (m/plant) was calculated from weighted mean root densities and stand counts taken at the first sampling time.

Results. In 1992 and 1993, yield and total dry matter (TDM) production at the low fertility sites were significantly increased by K application (**Table 1**). There was no significant interaction between plant population and management of K fertilizers for yield or TDM, suggesting that no adjustment in fertilizer management is required for higher plant populations. With respect to root measurements, a trade off was observed between root density and root length per plant at different plant populations, with lower root density but higher root length per plant observed at lower plant populations (data not shown). When K uptake per plant was divided by mean root length per plant, the K uptake per unit root length was not affected by plant population except in one of 8 cases (**Table 2**). Thus, since there was no difference in K uptake per unit root length, the amount of K uptake was proportional to root length, with more K uptake per plant at lower plant populations, but less overall K uptake per hectare.

When we examined effect of broadcast K on root density at the low K sites we found higher root length density with K fertilization at both growth stages only in 1993 (**Table 3**). In 1992, the same trend was evident, but differences were not significant due to much greater variability in the root length measurements. These two crop years also showed a yield response to K at the low K site (**Table 1**). Temperatures were lower in 1992, and 1993 was much wetter than the 30-year averages (**Table 4**). In 1991, there was no fertilizer effect on root measurements or yield. This result may have been due to higher soil K at that site, better growing conditions, and 5 to 10 times higher root densities. In 1992 and 1993, when there was a response of root length density to both K fertilizer treatments, application of the lower rate (93 kg K/ha) usually maximized root length density (**Figure 1**).

Table 2. Effect of plant population on K uptake per unit root length analyzed separately for each year, growth stage, and site. Mean ± standard deviation.

Site	Year	Growth stage	Plants per ha 56,800	Plants per ha 81,500	Level of significance
			----- μ K/cm root -----		
Low K	1991	V 7-8	195 ± 125	175 ± 63	NS
	1992	V 5-6	166 ± 100	86 ± 36	0.02
	1993	V 5	167 ± 90	149 ± 41	NS
	1993	V 8	814 ± 269	913 ± 419	NS
High K	1991	V 7-8	301 ± 37	300 ± 38	NS
	1992	V 5-6	1212 ± 423	1265 ± 564	NS
	1993	V 5	326 ± 90	329 ± 64	NS
	1993	V 8	983 ± 320	1320 ± 300	NS

Table 1. Effect of K management on yield and total dry matter production in 1991, 1992 and 1993.

	Yield			Total dry matter production		
	1991	1992	1993	1991	1992	1993
	---------- Mg/ha ----------					
Low fertility sites						
No K	11.07	7.16	7.33	20.65	13.98	12.23
37 kg K/ha (starter)	10.93	8.50	7.91	21.68	15.19	14.07
93 kg K/ha (broadcast)	10.97	8.90	7.58	21.50	15.99	13.37
186 kg K/ha (broadcast)	11.34	8.94	7.76	21.24	15.93	14.02
LSD (0.05)	NS	0.47	0.30	NS	1.12	0.81
High fertility sites						
No K	11.16	9.31	8.78	19.98	17.52	16.11
14 kg K/ha (starter)	11.08	9.46	8.67	20.99	16.82	15.91
Significance	NS	NS	NS	NS	NS	NS

The only exception was for 1993, when the 186 kg/ha rate increased root density for the high plant population but not for the low plant population, resulting in a significant interaction of plant population and fertilizer rate (P=0.002). Similar trends occurred for root length (data not shown).

Table 3. Weighted mean root density as affected by rate of broadcast K in 2 years at two growth stages at the low fertility site.

	1992		1993	
Broadcast K	V 5-6	V 7-8	V 5	V 8
kg K/ha	cm/cm^3			
0	0.13	0.18	0.12	0.12
93	0.21	0.25	0.18	0.19
186	0.18	0.22	0.18	0.20
LSD	0.12	0.12	0.03	0.03
CV	56%	45%	23%	16%

Table 4. Temperature and precipitation data for growing season in 1991-1994 and 30-year averages at Morris, MN.

	1991	1992	1993	1994	1961-1990
Mean monthly temperature (°C)					
April	7.8	3.9	6.1	6.7	5.6
May	15.6	15.6	13.3	15.6	13.3
June	21.1	17.2	16.7	20.0	18.3
July	21.1	17.2	20.0	20.0	21.1
August	21.1	17.2	20.0	18.9	19.4
Mean	17.3	14.2	15.2	16.2	15.5
Mean monthly precipitation (mm)					
May	89	37	157	28	70
June	142	120	139	64	95
July	160	141	201	157	87
August	70	37	72	55	83
Mean	115	84	142	76	84

With regard to effects of K fertilizer on root distribution, there were no effects in 1991, but in 1992 and 1993, fertilizer K usually increased root density at all positions. This non-localized effect is consistent with reports mentioned previously[4,5]. There were only two occurrences of significant interactions between fertilizer treatment and root length density at different depths and distances from the row. In 1992 (data not shown) and 1993 (**Table 5**) at the earlier growth stage, more roots were present at greater depths and in inter-row positions with K fertilizer applied. Thus it appears that addition of K fertilizer allowed the root system to exploit a greater soil volume more quickly under the cool (1992) or wet (1993) conditions which occurred in those years.

Early Season Potassium Deficiency in Ridge-Tilled Corn

Potassium deficiency is a common problem in ridge-till or no-till corn production in Minnesota as well as Iowa and South Dakota[9] and can result in reduced corn yields. The deficiency occurs even on soils with high to very high soil test K values,

Table 5. Mean root length densities at V5 for the low K site in 1993. Interaction of fertilizer treatment x sample distance from row was significant at P=0.05.

	Distance from row		
Broadcast K, kg/ha	7.5 cm	15 cm	30 cm
	cm/cm^3		
No K	0.35	0.063	0.011
93	0.42	0.108	0.025
186	0.40	0.113	0.039
LSD (0.05)	0.10	0.030	0.013

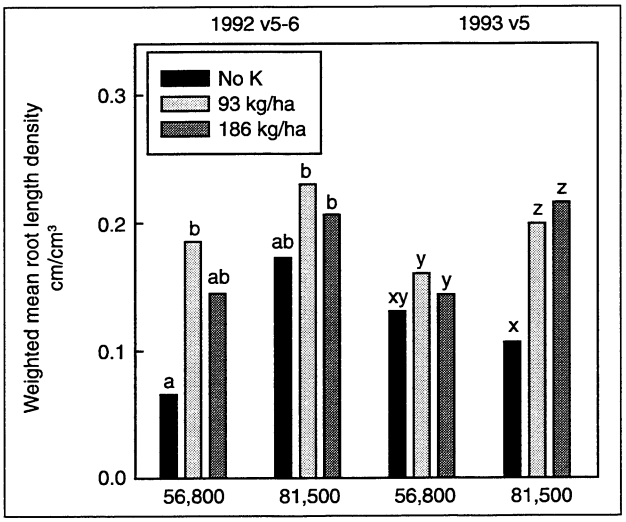

Figure 1. Mean root length densities at two plant populations and three K application rates in 1992 and 1993 at the first sampling time. For 1992 and 1993, P values for the fertilizer rate by population interaction were 0.89 and 0.05, respectively.

and severity of the deficiency varies with hybrid[1,9]. Band application of about 40 kg K/ha can correct the problem[9]. The purpose of this research was to identify the physiological basis for this problem by examining the development and morphology of root systems for hybrids which exhibit substantial differences in severity of K deficiency symptoms.

Methods. This study was conducted from 1992-94 at the West Central Experiment Station at Morris, MN on Tara silt loam soil (Pachic Udic Haploboroll) which had been under ridge tillage management for over 10 years. Three factors (tillage system, hybrid, and K treatment) were combined in a complete factorial with four replications. A split-split plot arrangement was used with tillage system (fall chisel, ridge-till) as the main plot. The split plot factors were hybrids (Pioneer 3732 and 3737) and fertilizer treatment (0 or 37 kg K/ha band applied or broadcast). Potassium was applied in late October each year along with anhydrous ammonia at the recommended rate.

Roots were measured by two methods. Root density was measured using sample cores collected at two sampling times (V4 and V7) in the control plots only (no K applied). At V4, root core samples were collected 5 cm away from growing plants within the row and perpendicular to the row and subdivided into 0 to 7.5 cm, 7.5 to 15 cm, and 15 to 30 cm

increments. At V7, cores were taken within the row (5 cm from plant) and at 7.5, 15, and 30 cm from the row. Cores were divided into 3 depth increments (0 to 15 cm, 15 to 30 cm, and 30 to 60 cm). Four complete sets of cores were taken per plot and composited before processing as described earlier.

The second method used to estimate root activity was a tracer method[1] involving the measurement of plant uptake of the non-essential cations, strontium (Sr), rubidium (Rb), and lithium (Li), injected at 7.5, 15 and 30 cm depths at a distance of 10 cm from and perpendicular to the row. Plant samples were collected at V4 and V7, dried, weighed, ground, and analyzed for uptake of K and all three tracers by atomic absorption spectroscopy. To estimate root activity, total uptake of the tracers was calculated on a per plant basis. Amounts of these non-essential ions taken up by control plants were subtracted to obtain net uptake due to the presence of the injected tracer. To convert all the tracer uptake values to a common measure (net Rb uptake per plant), we established a plot in the border where the same amounts of each ion were injected at the same depth to determine ratios of uptake of Rb/Sr and Rb/Li.

Results. Yields measured in 1992 and 1993 were approximately one-half of those measured in 1994 due to poor growing conditions. Grain and silage yields are shown in **Table 6**. There were significant effects of tillage and hybrid on yield in 1992, but no interaction. In 1993, when only silage could be harvested, there were no significant differences between tillage or hybrid treatments. While tillage did not affect yield in 1994, hybrid yields differed, and a significant interaction of hybrid and tillage treatment occurred.

Table 6. Grain and silage yields for all three years of the study. Only silage could be harvested in 1993. ANOVA results are significant at the 0.05 (*), 0.01 (**) and 0.001 (***) levels or not significant (NS).

	Grain yield		Silage yield	
Treatment	1992	1994	1993	1994
	Mg/ha			
Chisel				
3732	5.8	11.0	8.9	20.1
3737	6.5	11.5	9.7	20.8
Ridge				
3732	4.3	10.1	8.1	21.1
3737	4.8	11.9	8.6	21.4
Tillage	*	NS	NS	NS
Hybrid	**	***	NS	NS
T x H	NS	*	NS	NS

In 1994, which had excellent growing conditions (**Table 4**), dry weights of the plants were not different at the V4 stage, but there was significantly more K taken up by Pioneer 3737 compared to 3732 in the ridge-tillage planting system (**Figure 2**). At later growth stages (V7 and V12) there was a significant tillage by hybrid interaction for both K uptake and dry weight (data not shown). Not only was K uptake higher for 3737 compared to 3732 in the ridge-till treatment, but uptake of uninjected soil Rb was higher as well (**Figure 3**). In all cases, ridge tilled 3732 had the lowest rate of ion influx per unit root length of any of the treatment combinations. While these differences could be due to physiological differences in membrane uptake for 3732 compared to 3737, this seems unlikely, since uptake for the two hybrids was comparable for the chisel treatment.

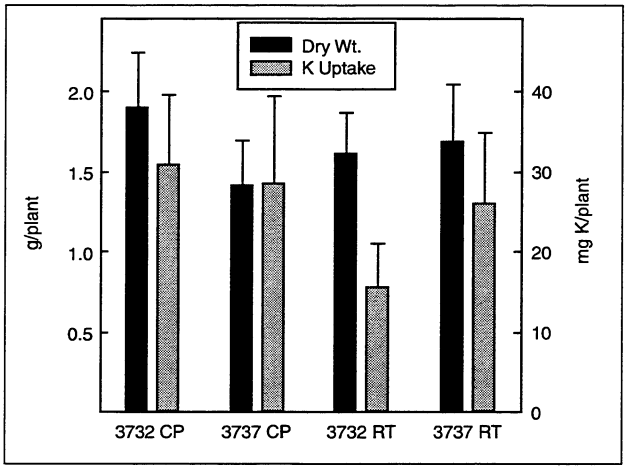

Figure 2. Shoot dry weight and K uptake by plants at the V4 growth stage in 1994. Values shown for both hybrids (Pioneer 3732 and 3737) and both tillage systems (CP = chisel plow, RT = ridge-till). Error bars show standard deviations from the mean.

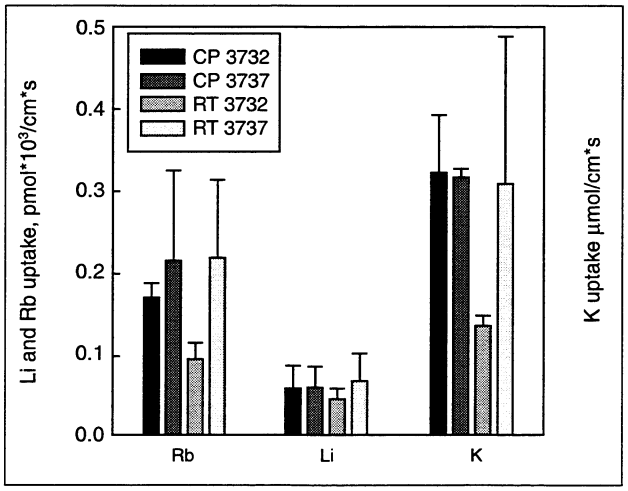

Figure 3. Ion influx of noninjected soil Rb, Li and K in 1994 for two hybrids (Pioneer 3732 and 3737) and two tillage systems (CP = chisel plow, RT = ridge-till). Error bars show standard deviations from the mean.

Data from both root measurement techniques suggest that differences in root architecture may play a role in the hybrid differences in K uptake. As shown in **Table 7**, Pioneer 3737 had more of its roots and higher root activity in the surface layer (0 to 15 cm) compared to 3732, although total root density and activity were quite similar for the two hybrids. Pioneer 3732, in contrast, had greater amounts of root length and activity below 15 cm.

Since K tends to accumulate near the soil surface with reduced tillage[8, 10], this tendency of 3737 to have increased root density and activity at the surface may impart an advantage for K uptake.

Fertilizer application increased K uptake in all three years (**Figure 4**) with placement significant only in 1992 and 1993. Only in these two years were yields and plant dry weights affected by fertilizer treatment. Effects of placement on root activity are shown for the V7 growth stage in 1992 in **Figure 5**. In this case, banded K increased root activity at all three injection depths, although more typically significant increases were observed only at the 15 and 30 cm depths (**Table 8**). These differences in root activity were significant only at the earlier growth stages (V4 and V7). By the V12 sampling date there were no significant differences due to K addition or placement.

Table 7. Hybrid differences in percent distribution of root length and root activity with depth at V7 in 1994. Root length estimated from root core data was 221 m/plant for 3732 and 227 m/plant for 3737. Root activity estimated from tracer technique was 5.19 mg net Rb uptake/plant for 3732 and 5.66 mg net Rb uptake/plant for 3737.

Hybrid	Depth	Root length	Root activity
	cm	- - - - - - - - % - - - - - - - -	
3732	0 - 15	48	35
	15 - 30	42	61
	30 - 60	10	5
3737	0 - 15	63	52
	15 - 30	32	45
	30 - 60	5	3

Table 8. Analyses of variance for tracer uptake (mg net Rb uptake per plant) at growth stage V7 in response to K addition and K placement.

Source	Injection depth, cm		
	7.5	15	30
	- - - - - - - - Probability - - - - - - - -		
K addition			
1992	0.924	0.029	0.044
1993	0.966	0.882	0.001
1994	0.911	0.847	0.379
K placement			
1992	0.079	0.009	0.001
1993	0.928	0.063	0.024
1994	0.583	0.714	0.567

Summary

Although a review of the literature suggests that K management has little effect on root growth and distribution, our results indicate that when conditions are K deficient and poor for growth, K application can result in significant increases in root extension at early growth stages. This increased growth throughout the root system allows the plant to exploit a greater soil volume for nutrients and water.

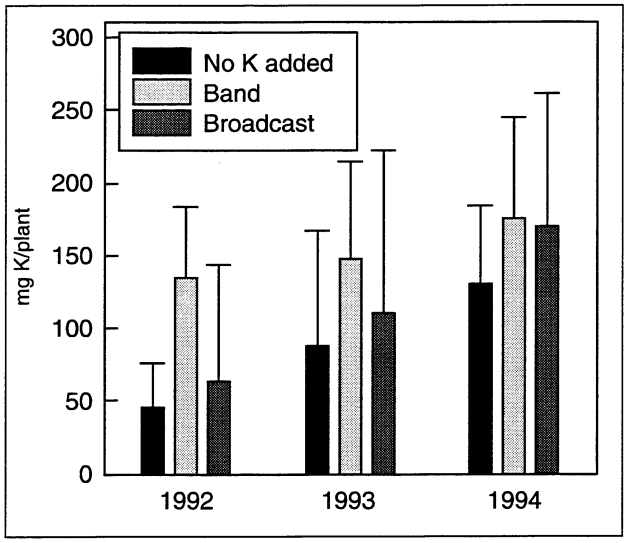

Figure 4. Potassium uptake per plant at V7 for three K treatments for three years, 1992-1994. Error bars show standard deviations from the mean. ANOVA probability values for K addition are 0.0001, 0.001 and 0.005 in 1992, 1993 and 1994, respectively. For K placement, P values are 0.0001, 0.05, and 0.69 for the same years.

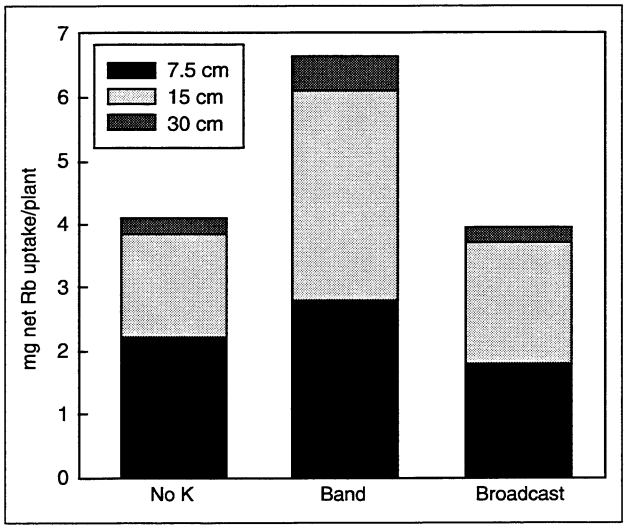

Figure 5. Effects of K treatment on root activity in 1992, measured by uptake of injected tracer from three depths.

In the plant population study, we found that K fertilization generally increased root density and length in all positions. Where interactions occurred, the greatest effects of K addition were at deeper and inter-row positions. The effect of K fertilizer on roots was greater when growing conditions were poor, as in the cool, wet summers of 1992 and 1993.

With respect to tillage, hybrids with more roots near the surface were better adapted to the ridge tillage planting system. As in the population study, root system activity in the cool (1992) and wet (1993) years was most responsive to K addition. Banding increased root activity, especially at 15 and 30 cm depths, compared to broadcast application. Hybrid differences in tracer and K uptake were much larger than differences in root length

density, suggesting that reduced tillage conditions inhibit uptake beyond the effects on root growth, either by affecting root influx rate or soil availability of K.

Synopsis of Future Research Imperatives

One intriguing conclusion from these studies is that differences in hybrid root architecture may play a significant role in crop productivity. From the research reported here, we are still not certain how much of the observed hybrid differences in K uptake and performance in the ridge till system are due to genetics which control root system geometry, membrane uptake differences, or other factors. Can more awareness of such hybrid differences improve our crop management strategies, especially with respect to tillage and fertility management?

Our work also points to questions about changes in soil K availability in reduced tillage planting systems. Is this simply because of reduced root function in these systems, or is K chemistry in soil modified by physical differences in the ridge system? Are present soil extractants appropriate for reduced tillage systems? Should we be recommending a sampling depth other than the top 15 cm? Answers to these questions can help improve management and increase adoption of reduced tillage practices.

Acknowledgments

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Chapter 14:
Role of Foliar Potassium and Root Hairs in Uptake of Potassium by Plant Roots

J.K. Keino, C.A. Beyrouty, D.M. Oosterhuis, and E.E. Gbur

Department of Agronomy, University of Arkansas, Fayetteville, AR 72701

Introduction

Potassium (K) is second only to nitrogen (N) as the most limiting mineral nutrient for cotton (*Gossypium hirsutum* L.)[30]. Shortage of K can dramatically reduce cotton yields and quality. Widespread K deficiencies have occurred across the U.S. Cotton Belt during mid-season even with preplant K applications[11]. These deficiencies have been attributed to high-yielding, fast-maturing cultivars[40], high K utilization for fruit development[44], a decrease in root growth as a result of partitioning to the fruit[10, 27], and soil-K fixation. In recent years, foliar application of K has been used to correct mid-season deficiencies[33, 38, 41] and has become a recommended practice in some states.

Root Development and Potassium Uptake

The efficiency of K uptake is associated with the ability of the plant to absorb K from soil solution. Root proliferation has been shown to increase K uptake[7] by increasing the root surface area (RSA) exposed to K in solution.

Nutrient absorption by plants occurs in three steps[1]: 1) movement of a nutrient to the root surface by mass flow and diffusion, 2) translocation of the nutrient from the exterior to the interior of the root, and 3) transport of the nutrient to the shoot. Absorption of nutrients by roots from an external medium can be either passive through the apoplast or active through the symplast. Movement of ions through the apoplast occurs in the transpiration stream without energy expenditure[16], while movement through the symplast requires the expenditure of metabolic energy[24]. The absorption and translocation capabilities of roots for K may become segregated along the root axis because of biochemical and anatomical changes which occur as tissues in the root develop[13].

Potassium uptake by plants is highly affected by the concentration of K external to the root. Potassium uptake in low solution concentrations is governed by saturation kinetics[16]. An increase in external concentration increases K influx into roots asymptotically until a maximum uptake is achieved, where efflux starts to occur[4, 32]. Maximum influx occurs in soil solution when the external concentration reaches an equilibrium with the internal root concentration. Maximum influx rate changes with changes in plant growth, shifts in dominant sinks of assimilates and the external solution concentration[5].

Nissen[36] evaluated barley (*Hordeum vulgare* L.) roots of low and high K content and found that under low external K concentration, roots with low K content had greater uptake rates of K than those with high K content. Glass[20] attributed these differences in uptake to differences in plasmalemma influx isotherms which differ for the low and high K barley plants. Several studies have suggested that K absorption by roots is mediated by a high-affinity system which predominates at low K external concentration[16, 45].

Plant roots can regulate internal K concentration regardless of plant genetics[22, 37]. However, the accumulation of K in root cells occurs at the expense of metabolic energy from photosynthetic activity[29]. A simple model showing K ion transport into the roots was developed by Pettersson and Jensen[42]. Potassium passes through the free space of root cells before reaching the plasmalemma. In the plasmalemma, K is transported through the tonoplast and accumulates in vacuoles[19]. The K carrier in the plasmalemma appears to be allosterically regulated by K when the intracellular K concentration in the roots increases beyond the external solution concentration[21]. Wild et al.[51] found that with an increase in K internal concentration of roots, there was a decrease in K uptake by a factor of 3 to 6. Other models proposed to explain active uptake of nutrients are described by Nissen[35].

Foliar Application

Foliar application of nutrients is used in crop production as a quick and efficient way to correct nutrient deficiencies in plants. Nutrients applied to leaves are considered to be more readily available to the plant at concentrations lower than when soil applied. The ability of plants to absorb nutrients through leaves depends upon nutrient penetration through the cuticle[28] and epidermal cells and is dictated by the age of the plants, water status of the leaf, and time of application[53]. Other important factors that may affect foliar uptake are the size and molecular weight of the solute used, as well as concentration and volatility of the substance[8, 39], light intensity, and plant internal concentration[32].

The uptake mechanism by leaves is not well understood, but several studies have shown that absorption of ions by leaves follows dual patterns[26, 50], possibly representing dual mechanisms similar to that of root uptake. Although very little is known about the effect of foliar applied nutrients on up-

take of nutrients by roots, Thorne[49] observed an increase in K uptake by roots of sugar beets (*Beta vulgaris* L.) as a result of painting the leaves with a KCl solution.

Role of Root Hairs in Nutrient Uptake

Root hairs are outgrowths of epidermal cells that have undergone morphological transformations with no change in physiological function[9]. They are active for 2 to 8 days and are mostly unicellular structures with an average range in length of 80 to 1,500 µm and a diameter of 5 to 15 µm[6, 15]. Root hairs enhance water and nutrient uptake capacity of plants by increasing the root absorptive surface area. An increase of four to eight times in root surface area[6, 14, 43] and a 20-fold increase in total root length[23] of several plant species have been reported to be associated with the presence of root hairs.

Root hairs play a significant role in K influx into roots. Gassmann and Schroeder[17] used isolated protoplasts from root hairs of wheat (*Triticum aestivum* L.) to study K uptake and found that the protoplast provided a mechanism by which K ions can be taken up by root hairs. Differences among plant species in root hair length and number have been reported to affect uptake of nutrients. Genotypical differences in root hair length are important for the concentration gradient of K around the root[32]. Itoh and Barber[25] studied six plant species for phosphorus (P) uptake kinetics as influenced by root hairs. The six species selected differed in root hair morphology. The contribution of root hairs to P uptake was greatest for Russian thistle (*Salsola kali* L.) which had the longest root hairs, thus the greatest increase in root surface area. Tomato (*Lycopersicum esculentum* L.) had the next largest root hair surface area followed by lettuce (*Lactuca sativa* L.).

Although the use of foliar K in cotton has become a standard practice in Arkansas, yield results have been very inconsistent. Currently, little is known about the effect of foliar K application or root hair formation on K uptake by cotton roots. An understanding of these effects may provide insight into the inconsistent growth and yield responses to foliar K by field-grown cotton. Consequently two studies were conducted to 1) evaluate the effect of foliar K application on kinetic uptake parameters of two cotton cultivars and 2) determine if differences in uptake kinetics might be influenced by root hair development.

Materials and Methods

Foliar potassium study

Kinetic uptake parameters of cotton were determined using the nutrient depletion technique of Claassen and Barber[12]. Two cotton cultivars were selected to represent cultivars commonly grown in Arkansas that differ in maturity and root growth dynamics under field conditions[27]. The cultivars selected for the study were the early maturing 'Deltapine 20' (DP20) and the late maturing 'Deltapine 90' (DP90). The experiment was conducted in a controlled environmental chamber with day/night temperature of 30°C/25°C, 14-hour photoperiod, and irradiance of 550 µmol photon/m^2/s at the top of the canopy.

Five days after germination (DAG) on moist paper towels, two seedlings of each cotton cultivar were transferred into 4-L pots containing nutrient solution with the following elemental concentration; 3.20 mM NO_3-N, 1.0 mM K, 1.0 mM P, 1.5 mM Ca, 1.0 mM Mg, 1.00 mM SO_4-S, 89.5 µM Fe, 0.21 µM Mo, 46.3 µM B, 3.7 µM Mn, 0.77 µM Zn, 0.32 µM Cu, 7.3 µM Cl. The solution was replaced after 5 days and every 2 days thereafter and the pH was maintained at 6.0 to 6.5 using 1 M NaOH buffer solution.

Four days prior to K depletion, the two 21-day-old-plants per pot were transferred to K-free nutrient solution and foliar K treatment was applied with brushes. A solution of 1.0 M KNO_3 was applied foliarly by painting the leaves with 1.26 mL of solution per plant (equivalent to 10 kg KNO_3/ha, the recommended field application) and the control treatment was painted with an equivalent amount of double deionized water. At this time, both cultivars were initiating reproductive development (formation of floral buds or squares).

To start the depletion the paired cotton plants were transferred into 2-L pots containing nutrient solution amended with 80.0 µmol K/L as KNO_3. Solution was sampled continuously with a fraction collector every 30 minutes for a period of 13 hours at a rate of 0.23 mL/min. Potassium concentration of the sampled solutions was determined by atomic absorption.

At the end of the depletion, shoots and roots were harvested. The leaves were rinsed and leaf area and fresh root weight determined. Roots were stained with 2 mL of toluidine blue 0 (1 percent), and root length (RL) was calculated by the line-intercept method[46]. Shoot and root samples were oven dried and weighed. Average root radius (r_0) was calculated as $r_0 = (\text{fresh weight}/\pi L)^{0.5}$ [3]. Root surface area was calculated from the equation $RSA = 2r_0\pi L$, assuming the root is a cylinder[3].

For each foliar-cultivar treatment combination, a depletion curve was developed as described by Claassen and Barber[12]. The relation shown in the following equation was fitted to the data of K remaining in solution, y, (µmol/L) vs. time, x, (min.) as described by Gbur and Beyrouty[18].

$$x = \beta_0 - \beta_1(y - C_{min}) - \beta_2 \ln(y - C_{min})$$

where:

β_0=integration constant,

$\beta_1 = V/(RSA * I_{max})$,

$\beta_2 = (K_m * V)/(RSA * I_{max})$,

V=initial solution volume (L),

RSA=root surface area (cm^2).

C_{min} was estimated visually from the depletion curves, and I_{max} and K_m were calculated from the following equations:

$$I_{max} = V/(RSA^*\beta_1),$$

$$K_m = \beta_2/\beta_1.$$

I_{max} = maximal influx at high concentration of the nutrient in solution, (nmol/m²/s).

K_m = concentration where uptake velocity is ½ I_{max} (µmol/L), and describes the affinity for a nutrient by a plant. The higher the K_m, the lower the affinity for the nutrient. C_{min} = estimate of the lowest nutrient concentration where uptake ceases (µmol/L).

The experiment was conducted in a randomized complete block design with three replications and a 2 x 2 factorial structure (cultivars and foliar treatment). Analysis of variance was carried out for all plant and kinetic parameters. Fisher's protected LSD (P=0.05) was used to separate means when appropriate. All statistical analyses were carried out using SAS Version 6.12 (SAS Inc. Cary, NC).

Root hair study

Twelve seeds of DP20 and DP90 were placed in moist paper towels for seven days in a growth chamber. Total tap root length was measured and the portion of tap root characterized by the presence of root hairs was determined with a microscope with a 4x objective lens. This zone of root hairs was divided into two equal sections (**Figure 1**). Each root section was stained with 0.1 percent Calcofluor and mounted in 25 percent glycerin with phenol crystal on a microscope slide. Five longitudinal subsections, each measuring 23.6 by 472 µm (WxL), were randomly selected from each section of root hairs for measurements. Root hair numbers were determined for each sub-section, and average length of root hairs was measured with an ocular micrometer. Values reported for each root hair section of the tap root were averages of the five subsections.

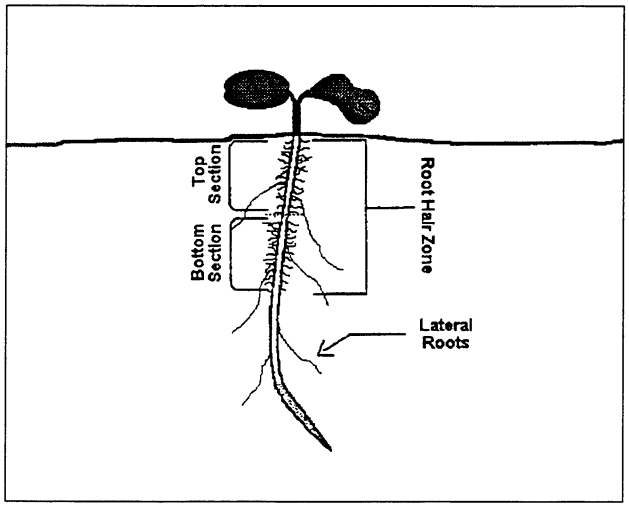

Figure 1. Diagram of a cotton seedling showing the root hair zone where measurements were collected.

The experiment was analyzed as a split plot in which the whole plot structure was a randomized complete block design with three replications and two cultivars. Root hair section (top and bottom) was the split plot factor. All statistical analyses were carried out using SAS version 6.12 (SAS Inc. Cary, NC).

Results and Discussion

Foliar study

Depletion curves for foliar and non-foliar treated DP20 and DP90 cultivars are presented in **Figure 2**. Comparing plants that did not receive foliar K, the late maturing DP90 absorbed K from solution slightly more rapidly than the early maturing DP20. The average depletion rate for the first 210 minutes of rapid depletion was approximately 0.24 and 0.31 µmol/L/min for DP20 and DP90, respectively. This reflects an 18 percent larger root surface area of DP90 compared to DP20.

After 210 minutes, depletion of K from solution effectively ceased for all treatments. The application of foliar K to DP20 resulted in 27 percent greater depletion of K from solution than its non-foliar K control (**Figure 2**). In contrast, the minimal concentration of K in solution for DP90 was similar with or without foliar K.

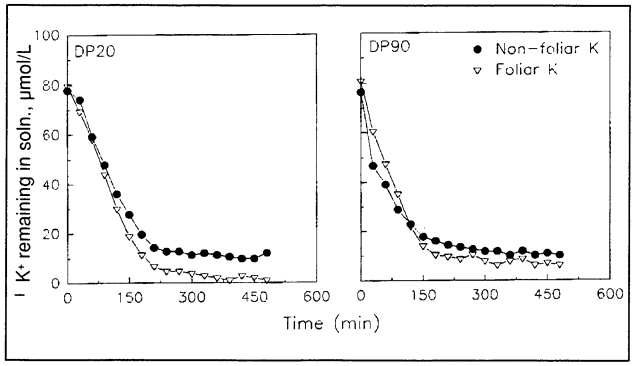

Figure 2. Potassium depletion by DP20 and DP90 cotton cultivars as affected by foliar K application. The curves are averages of three replications per cultivar and treatment.

Effect of foliar potassium on kinetic parameters

The kinetic parameters that describe K uptake by DP20 and DP90 are presented in **Table 1**. Based on the analysis of variance for the kinetic uptake parameters, there were significant cultivar differences for I_{max} and C_{min} but not for K_m. The I_{max} for DP20 was about 3.5 times greater than for DP90. However, the total root surface area for DP90 was 18 percent higher than for DP20. Therefore, it is speculated that the larger value for I_{max} of K for DP20 could possibly have resulted from a greater number of active sites along the root axis or greater root hair surface area which is not accounted for in the line-intercept method.

Table 1. Kinetic uptake parameters for K by two cotton cultivars averaged over treatments.

Cultivar	I_{max}	K_m	C_{min}
	Nmol/m²/s	- - - - - - - - µmol/L - - - - - - - -	
DP20	23.1 a†	19.3 a	9.3 a
DP90	6.5 b	10.1 a	2.7 b

† Means followed by the same letter are not significantly different (0.05).

Although no significant differences between treatments resulted from foliar application for any of the kinetic parameters, there was a trend for an increase in I_{max} for DP20 and DP90 when foliar K was applied (**Table 2**).

Table 2. Kinetic uptake parameters for K by two cotton cultivars treated with or without foliar K.

Cultivar	Treatment	I_{max}	K_m	C_{min}
		Nmol/m²/s	- - - - - µmol/L - - - - -	
DP20	No-foliar	15.7	15.6	12.8
	Foliar	30.5	23.0	5.8
	SE†	6.5	4.7	2.8
DP90	No-foliar	4.1	6.7	2.2
	Foliar	8.7	13.5	3.2
	SE	1.5	1.0	0.7

†Standard error.

These higher values of I_{max} suggest that foliar application stimulated the absorption of solution K by roots possibly by altering source-sink relationships. Thorne[49] found that painting the leaves of sugarbeet plants with KCl solution increased K uptake by roots from solution.

Potassium plays a significant role in plant enzymatic reactions and cell metabolism. It stimulates translocation of sugars from leaves into roots by increasing the rate of CO_2 assimilation and promoting ATP synthesis[24, 31]. This CO_2 assimilation increases photosynthates and the energy (ATP) required for phloem-loading[31]. The higher rate of phloem-loading increases the assimilate content in the sieve tubes of the leaves (source), thus decreasing the osmotic potential of the leaves and promoting water and nutrient uptake by roots.

The K_m values for the two cotton cultivars are reported in **Table 2**. These values reflect an apparent increase in solution K concentration needed to achieve ½ I_{max} for both cultivars when foliar K was applied. The increase in I_{max} and K_m in response to foliar K application suggests that foliar K may stimulate development of more sites for active uptake of K while decreasing the affinity of K by these sites.

Foliar application to DP20 resulted in an apparent decrease in C_{min} of 55 percent, while increasing C_{min} of DP90 by 45 percent. Thus, application of foliar K to the early maturing DP20 apparently increases the ability of these plants to absorb K from lower solution concentration. Under very low K soil solution concentrations, application of foliar K to the DP20 cultivar can increase the ability of the plant to take up nutrients present at lower concentration when compared to the non-foliar treatment. However, foliar K application to DP90 decreases its ability to take up nutrients present in lower solution concentrations when compared to the non-foliar treatment.

Net K influx (ln)(µmol/m/s) curves were constructed (data not shown) with changes in solution concentrations (C) for the two cotton cultivars by using the Michaelis- Menten equation[12].

$$In = [I_{max}(C-C_{min})]/[K_m + C - C_{min}]$$

These curves can be used to predict net K influx at any solution K concentration. For example, the average solution concentration obtained in a Crowley silt loam (fine, montmorillonitic, thermic Typic Albaqualfs) at 0-10 cm prior to flooding was 112 µM K/L[47]. When this concentration is imposed onto the influx curves, we find that the net influx rate of K for this soil under cotton treated with and without foliar K was 83 and 87 percent of I_{max}, respectively for DP20 and 92 and 95 percent of I_{max}, respectively for DP90. Barber et al.[2] found that 43 percent of 142 Midwestern soils contained K solution concentrations less than 128 µM K/L. Based on this concentration, the resultant net influx of K would be similar to those above for DP20 and DP90. It would appear that under normal field conditions, K solution concentrations are less than that needed to achieve I_{max} for either cultivar.

It would appear from the results from this study that rather than reduce K uptake by roots, foliar application of K stimulates more rapid uptake of soil K which may result in quicker depletion of limited K reserves, especially from soils with low K buffering capacity.

Effect of foliar K on plant parameters

Although no significant treatment differences were observed among plant growth parameters, foliar K application appeared to alter plant growth within the 4 days following foliar treatments (data not shown). Total root length decreased by 2 and 7 percent for foliar treated DP20 and DP90 cotton cultivars, respectively as compared to non-foliar treated plants. The number of squares was increased by 8 and 14 percent following foliar K application to DP20 and DP90, respectively. The rapid development of squares in response to foliar K application could be attributed to increased nutrient uptake by roots. Bukovac and Wittwer[8] showed that foliar-applied labeled ^{42}K was rapidly absorbed and translocated to other developing parts of the plant. Thorne[49] also reported the importance of foliar feeding to prevent depletion of nutrients during grain-filling. Although the K concentration in shoot tissue for foliar-treated cotton plants was not significantly affected, there was an apparent increase of 6 and 14 percent in tissue K concentration for foliar-treated DP20 and DP90, respectively. Thorne[48] reported that spraying plants with solution con-

taining one nutrient increased the amount of that and other nutrients in the plant. This apparent increase in shoot tissue K is likely a combination of K absorbed by roots and leaves.

Evaluation of root hair growth

Several studies have reported that root hairs can increase the root absorptive area[25, 43] which may impact kinetic uptake parameters. We were interested in determining if differences in root hair development between the non-foliar treated cultivars could help explain cultivar differences in I_{max} for K as measured from the depletions. The average tap root length of 7-day-old plants was 147.9 and 94.6 mm for DP20 and DP90, respectively, of which 39 and 66 percent of the root length was occupied by root hairs. Similar results were reported by Roberts[43] who observed that 39 percent of the root surface area of seagrass *Halophila ovalis* (R. Br.) was covered by root hairs.

The average number of root hairs for each cultivar are presented in **Table 3**. Root hair number in both sections of the tap root where root hairs were found (**Figure 1**) was significantly greater for DP20 than for DP90. The higher number of root hairs by DP20 may have increased the effective nutrient and water absorptive root surface area. Thus, nutrient uptake capacity of DP20 is expected to be greater than of DP90 because of increased absorptive surface area.

Table 3. Root hair number per unit length of tap root in the top and bottom sections of the root hair zone of 7-day-old seedlings of two cotton cultivars.

Cultivar	Number of root hairs	
	Top†	Bottom
	------ per mm of tap root ------	
DP20	40 aA‡	29 aB
DP90	34 bA	26 bB

† Sampled region along the tap root (**Figure 1**).
‡ Means in the same column followed by the same lower case letter are not significantly different (P=0.05) using Fisher's protected LSD. Means in the same row followed by the same upper case letter are not significantly different (P=0.05) using Fisher's protected LSD.

There was no significant difference in the average length of root hairs between the two cultivars, with an average length of 0.202 mm for DP20 and 0.194 mm for DP90. These values are in the same range as those reported by Misra et al.[34] and Itoh and Barber[25] for several plant species in which the root hair lengths ranged from 0.04 to 0.6 mm. Root hair length was 64 percent longer on average in the top section of the root hair zone (0.246 mm versus 0.150 mm) but decreased significantly in the bottom section. The decrease in root hair length along the root axis has been shown in other plant species and is attributed to differences in cell age and development along the tap root[43, 52].

Evaluation of root hairs of several plant species have shown that root hairs play an important role in nutrient uptake[25]. Due to the complexity of evaluating root hairs, limited consideration has been given to the role of root hairs on K uptake. However, consideration of root hairs may be important to accurately estimate uptake parameters such as those calculated in this study.

Summary

The results of these studies suggest that foliar K application impacted the kinetic uptake parameters of the two cultivars. It would appear that foliar application of K acted as a priming agent to stimulate the rate of K influx by roots for both DP20 and DP90 cultivars which may result in quicker depletion of soil solution K. This might stimulate deficiencies, especially on soils with low K buffering capacity. The fact that cultivar differences in kinetic uptake parameters were measured might be one reason for the inconsistency in plant response to foliar K under field conditions. Our results also suggest that root hairs may contribute to K uptake by cotton. The non-foliar treated DP20 had a higher I_{max} than the non-foliar treated DP90, although root surface area for DP20 was smaller. Further investigation showed that DP20 had a greater number of root hairs per unit tap root length. Root hairs are not typically accounted for in the line intercept method for determining root length. However, our results suggest that they should be considered in uptake studies.

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Chapter 15:

Potassium Uptake by Crops During the Season

G.L. Mullins and C.H. Burmester

Auburn University, Dept. of Agronomy & Soils, 202 Funchess Hall, Auburn University, AL 36849-5412.

Introduction

Potassium in the form of K^+ is absorbed in large quantities by all plants. Nitrogen is the only nutrient required in larger amounts. Of the essential cations, K is the most important in terms of its roles in plant physiology. Potassium has been shown to play an important role in water relations of the plant, meristematic growth, photosynthesis and translocation of photosynthates, enzyme activation and stomatal movement[42, 44]. It is the most abundant cation in the cytoplasm and thus makes a major contribution to the osmotic potential of plant cells[42].

One characteristic of K is its high rate of uptake by plant tissues. A combination of a high uptake rate with a high plant requirement for K often results in plants requiring more K than a soil can supply. If a soil cannot meet the uptake needs of a plant, K deficiencies will develop. In order to prevent K deficiencies, a soil must be able to supply K as it is needed by the plant. Thus, the ultimate goal of a K fertility program is to ensure K fertilizer is applied at the proper rate and time so that a soil in a given management program meets the K needs of a given crop. Understanding the uptake characteristics and distribution of K in a plant under a given management system during the growing season is important for making effective nutrient management decisions. In this paper we will summarize some of the pertinent literature regarding the uptake of K during the growing season by some common row crops and how K uptake may be affected by management.

Total Uptake

Potassium accumulation by plants during the course of a growing season can be quite high as illustrated in **Table 1**. Total seasonal K accumulation is dependent on the crop species, environmental conditions, management and yield potential. Thus the values given in **Table 1** should be viewed as a general guide for total K uptake by crops. For example, in **Table 1** corn (*Zea mays* L.) was reported to accumulate 172 kg/ha of K. A review of selected research (**Table 2**) shows that total K accumulation by corn varies greatly from this value, depending on growing conditions and final yields. As a second example, Beaton and Sekhon[8] in their review article, showed that K uptake by wheat (*Triticum* spp.) may range from 34 to 189 kg/ha.

Crops also vary in the proportion of total accumulated K removed when the crop is harvested (**Table 1**). For forage crops, especially if cut and harvested as hay, essentially all of the K that is accumulated will be removed at harvest. For example, Rominger et al.[53] reported total K accumulation and removal by alfalfa (*Medicago sativa* L.) to range from 66 to 484 kg/ha. Corn has been reported to contain between 16 to 44 percent of the total K in the grain[27, 36, 55, 60] depending on yield level and harvest index. Wheat may remove 14 to 27 percent of the total K at maturity in the grain[18, 33, 46]. Soybean [*Glycine max* (L.) Merr.], in comparison, removes a greater proportion of the total plant K in the seed. Hanway and Weber[22] reported that soybean seed harvest results in an average removal of 56 percent of the total K, while Coale and Grove[12] reported that soybean seed at R7 accounted for up to 65 percent of the total plant K. Caole and Grove[12] also reported that the proportion of total plant K in the seed at R7 dropped from 64 to 38 percent when comparing K removal from low and high K fertility regimes, respectively. In cotton, between 16.5 to 29 percent of total plant K is contained in harvested seed cotton[6, 21, 47]. As illustrated in these selected examples, in most row crop production systems only a proportion of the total accumulated

Table 1. Potassium uptake and removal by selected crops.

Crop	Potassium uptake		
	Yield, Mg/ha	Total, kg/ha	Harvested % of total
Barley (*Horendum vulgare* L.)	2.2	40	10
Wheat (*Triticum* spp.)	2.7	47	14
Oats (*Avena sativa* L.)	2.9	89	14
Corn (*Zea mays* L.)	9.5	172	37
Sugarcane (*Saccharum officinarum* L.)	75	250	100
Cotton (*Gossypium hirsutum* L.)	1.7	67	14
Coastal bermudagrass (*Cynodon dactylon* L.)	20	250	100
Red clover (*Trifolium pratense* L.)	6	95	100
Timothy (*Phleum pratense* L.)	6	90	100

Mengel and Kirkby (1987); Eakin (1972).

Table 2. Total, seasonal K uptake by corn.

Grain Yield Mg/ha	K uptake kg/ha	Reference
6.40	128	Sayre (1948)
1.19 to 7.15	32 to 106	Hanway (1962a)
7.62 to 9.29	249 to 315	Hargrove (1985)
10.9 to 13.4	258 to 372	Karlen et al. (1987b)
16.3	386	Karlen et al. (1988)

K is actually removed at harvest, while a large proportion of the accumulated K is returned to the soil as plant residues.

Potassium Accumulation over a Growing Season

Potassium uptake during a growing season follows a sigmoid type curve (**Figure 1**). Shortly after germination K uptake is relatively slow as young plants begin to grow. This is followed by a very rapid period of uptake with a near linear relationship between K accumulation with time. The period of rapid K accumulation with time parallels the rapid growth rate and production of dry matter by the plants. A comparison of K uptake and dry matter as a function of time shows that K absorption precedes the production of dry matter in many crops (**Figure 1**). This difference is most evident in corn as shown by the data from Sayre[55] plotted in **Figure 1**. Similar findings have been reported for cotton[47]. Henderson and Kamprath[32] reported that for soybeans K uptake preceded dry matter production for about the first 60 to 80 days after planting. These data show that adequate available K will be needed early in the growing season to sustain the production of dry matter by a crop. It is during the respective peak uptake periods that adequate available K will need to be supplied to the plant to ensure high yields.

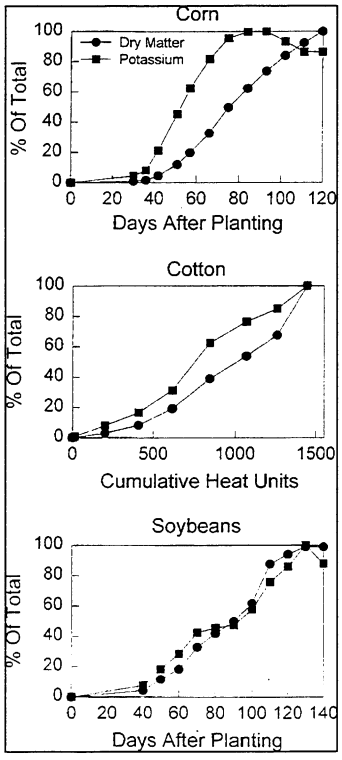

Figure 1. Percentages of the maximum amount of K accumulated and dry matter produced by corn[55], cotton[47] and soybean[32] at different times throughout the growing season.

During the peak uptake period, a substantial amount of the total seasonal K is accumulated by plants. For corn, peak uptake occurs during rapid vegetative growth prior to tasseling. Jordan et al.[34] reported that 59 percent of the total seasonal K uptake took place during a 21 day period between knee-high and tasseling. Hanway[27] reported that during the peak two week period from 38 to 52 days after emergence, corn accumulated 38 percent of the total K. Hanway[27] and Karlen et al.[36] reported that 75 and 86 percent of the total K, respectively, was accumulated by silking. Thus, K accumulation by corn is nearly complete by silking.

Similar results have been reported for wheat with the highest rate of uptake occurring between the end of tillering and the start of flowering. Gregory et al.[18] reported that the major period for K uptake by winter wheat occurred during the period of rapid shoot growth (mid-April to June).

Hocking[33] reported that by anthesis irrigated spring wheat had accumulated essentially 100 percent of the K that was present at maturity.

Hanway and Weber[23] observed the greatest accumulation of K in soybean between growth stages 5 and 9 (46 days) during which approximately 79 percent of the total K was accumulated. Harper[29] had similar findings and reported that the rapid increase in K uptake took place between 50 days of age and full bloom. In irrigated and non-irrigated soybean grown on a Coastal Plain soil, Karlen et al.[37] reported that approximately 62 to 72 percent of the total K was accumulated after the beginning of flowering. This range is similar to the findings of Batchelor and Scott[7] who reported that 65 percent of the total K was accumulated after flowering began in irrigated soybean.

Halevy[21] observed maximum K uptake in cotton at 72 to 84 days after emergence during which time one-third of the total K was accumulated. Bassett et al.[6] found that for irrigated cotton in California, 67 percent of the total K was accumulated during a six-week period (mid to late bloom) beginning July 1. Mullins and Burmester[47] reported that between 31 to 41 percent of the total K was accumulated during the peak 2-week uptake intervals (approximately mid-bloom) from 3-site years of non-irrigated cotton data.

The above results show that a majority of plant K is absorbed during a critical one-third of the plant growth cycle. In corn and small grains, the rapid accumulation period is during the rapid stage of vegetative growth, whereas in soybean the rapid uptake phase extends into the early reproductive stage (full bloom). For cotton, a majority of the K is accumulated during early to mid bloom stage.

Peak Daily Accumulation Rates

Peak daily K uptake rates can vary widely depending on crop and growing conditions. In their review article on corn, Welch and Flannery[60] reported that the average daily K uptake rate during the peak period ranged from 2.31 to 10.74 (7

references) kg K/ha/d. More recently, Karlen et al.[36] observed peak uptake rates of 7 to 10 kg/ha/d and Heckman and Kamprath[30] reported peak rates of 6.8 to 8.0 kg K/ha/d. Both of these studies were conducted on Coastal Plain soils in the southern U.S. while the studies in the review of Welch and Flannery[60] were conducted in the Midwest. Karlen et al[38] grew irrigated corn in the Southeastern Coastal Plain and calculated K accumulation rates by differentiating compound cubic polynomial equations. They reported peak accumulation rates ranging from 15 to 23 kg/ha/d which are much higher than values reported for Midwestern studies[60].

Peak uptake rates for soybean and wheat are lower than for corn. For soybean grown on a Coastal Plain soil in North Carolina, Henderson and Kamprath[32] observed an average peak uptake rate of 4.6 kg/ha/d (observed during early pod fill). Heckman and Kamprath[30] reported a similar peak accumulation rate for irrigated soybean of 5.2 kg K/ha/d from 49 to 77 days after planting.

Beaton and Sekhon[8] in their review article on wheat, stated that wheat accumulates K at rates up to 2.0 K/ha/d during the peak uptake or demand period at about midseason. Miller et al.[46] reported much higher rates of K uptake for hard red spring wheat. They analyzed K uptake data using compound cubic polynomials and found three peaks during the vegetative stage. The highest rate of 12 kg/ha/d occurred at 750 growing degree units (boot swollen).

For non-irrigated cotton in Georgia, Olson and Bledsoe[49] reported an average K accumulation rate of 2.8 kg/ha/d which occurred 90 to 105 days after planting. Mullins and Burmester[47] reported peak K accumulation rates during mid to full bloom ranging from 2.2 to 3.5 kg/ha/d for non-irrigated cotton in Alabama. For irrigated cotton, Halevy[21] reported a higher peak K accumulation rate of 4.6 kg/ha/d while Bassett et al.[6] reported peak accumulation rates for irrigated cotton to range from 2.1 to 3.4 kg K/ha/d.

In terms of nutrient management it is obvious that adequate levels of available soil K will be needed to supply the high demand of plant roots for K during peak uptake periods. Equally important, a producer needs to know when the peak demand period occurs in the life cycle of the crop being grown so that appropriate management practices can be used to ensure that adequate K is available during the critical uptake period.

Cotton is considered to be less efficient at obtaining K from the soil than many other plant species, and K deficiency occurs more frequently and with greater intensity as compared to many agronomic crops[39]. Gullick et al.[19] under greenhouse conditions concluded that cotton was less efficient at obtaining soil K as compared to barley (*Hordeum vulgare* L.). Corn has much higher daily uptake rates for K during the peak uptake period (2.31 to 23 kg/ha/d) compared to cotton (2.2 to 4.6 kg/ha/d), suggesting that the cotton root system is less efficient in recovering K from the soil. A comparison of published average daily K influx rates by root systems (µmol/m root/d) of corn[43], soybean[3] and cotton[56] when grown under field conditions verifies this hypothesis. All three tests were conducted on fertile soils. As illustrated in **Figure 2**, corn has very high K influx rates through approximately 50 days after planting. In comparison, cotton had relatively low average K influx rates throughout the growing season. Thus, the cotton root system appears to have a lower K uptake efficiency as compared to fibrous rooted crops which have higher root densities.

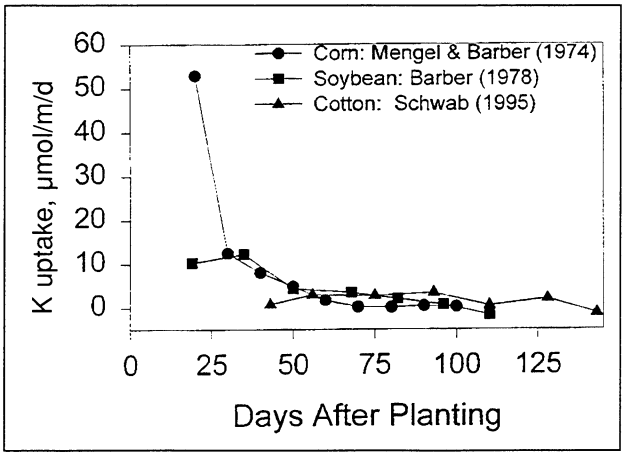

Figure 2. Average daily K influx rates (µmol K/m root/d) related to time after planting for corn[43], soybean[3] and cotton[56] roots growing under field conditions.

Distribution in Plant Parts

Early in the growth cycle of most crops a majority of the dry matter and absorbed nutrients are contributed to vegetative tissues. Later, reproductive tissues become the primary sinks for carbohydrates and nutrients. In some plants, developing reproductive structures become sufficiently strong sinks for carbohydrates and nutrients such that there is a redistribution or translocation of nutrients and carbohydrates from vegetative tissues to these organs. The degree of this translocation is dependent on the crop as well as K fertility status.

Examples of typical accumulation patterns for K by corn, wheat, soybean, and cotton are illustrated in **Figure 3**. For corn, a majority of the K is contained in vegetative tissues throughout the season (**Figure 3**). At maturity most of the K is present in vegetative tissues with a relatively small percentage in the grain[26, 34, 36, 55]. The dominant sink for K in corn throughout the season is the stem tissue. As mentioned previously, the grain typically contains about 20 percent of the total K at maturity[60]. Thus, corn harvested for grain will remove much less K than corn harvested for silage. Inspection of typical K accumulation curves for corn suggests that there is some translocation of K from

vegetative tissues to developing grain[26, 27, 34, 36, 55]. Some reports[36, 55] suggest that most of the translocation occurs from the stems. Potassium accumulation curves also show that most of the K accumulated by corn is taken up prior to silking. Although most authors have reported a continued but small increase in K uptake between silking and maturity, Sayre[55] and Jordan et al.[34] reported that K uptake ceased in August. Sayre[55] reported a decrease in plant K during late August-September. Potassium uptake and distribution by sorghum [*Sorghum bicolor* (L.) Moench] is similar to corn[54].

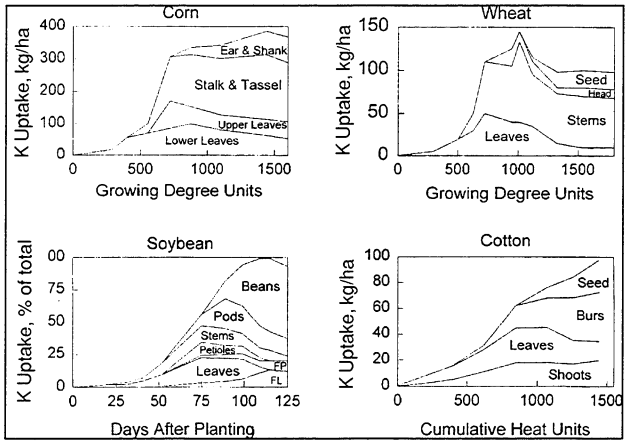

Figure 3. Accumulation and distribution of K in corn[36], wheat[46], soybean[23], and cotton[47] at various times during the growing season. Soybean data are the percentage of the maximum seasonal accumulation of K.

Wheat is similar to corn in that most K accumulation takes place prior to anthesis (**Figure 3**). However, wheat differs from corn in K accumulation patterns in that most authors report an actual loss of plant K after anthesis. Losses of plant K from anthesis to maturity have been reported to range from 20 to 60 percent[8, 17, 18, 46]. Gregory et al.[18] observed a 50 percent loss of the total plant K during the seven weeks between anthesis and maturity, with 80 percent of the loss occurring in the first week. Miller et al.[46] observed a 34 percent loss in plant K between anthesis and the soft dough stage. Losses of K after anthesis have been attributed to several factors including leaching from leaves, leaf fall and the efflux of K from wheat roots[8]. During grain development there is an apparent redistribution of K from vegetative tissues to the grain. Hocking et al.[33] calculated that 22 percent of grain K had been retranslocated from vegetative tissues.

Potassium uptake by soybean is also illustrated in **Figure 3**. Based on the data of Hanway and Weber[22] K uptake is slow during early growth and increased to stage R5, followed by a high and near linear uptake rate between stages 5 to 9, and was complete by stage 10. Henderson and Kamprath[32] and Karlen et al.[35] reported similar results. There is an accumulation of K in the leaves, petioles and stems up to stage 8 and in the pods between stages 6 and 8. Hanway and Weber[22] observed a loss of K from vegetative tissues after stage 8 and an accumulation in the developing seed. They concluded that by stage 8 approximately half of the K in the seed had been translocated from other plant parts. Henderson and Kamprath[32] and Karlen et al.[37] had similar findings. At maturity, approximately 60 percent of the total plant K is contained in the seed. One major difference between seasonal K accumulation patterns for soybean as compared to corn and wheat (**Figure 3**) is that in soybean there is substantial accumulation of K during flowering and seed development. Approximately 65 percent of the total K is accumulated after flowering in soybean, suggesting that the soybean plant is dependent on adequate levels of available K up through most of the reproductive stage.

Like soybean, cotton plants accumulate a substantial proportion of K after the start of flowering (**Figure 3**). Most of the K accumulated during flowering goes into developing fruit, with the bur fraction serving as a major sink for K. Cotton plants have been reported to contain from 40 to 70 percent (typical values of about 55 to 60 percent) of the total K in the bolls at maturity[6, 21, 47, 49, 59]. At maturity the cotton plant contains about 20 percent of its total K in the seed and lint. Thus, only a small proportion of the total accumulated K is removed at harvest while the remaining K (approximately 80 percent) is returned back to the soil as plant residues. During the season there is some translocation of K from the leaves to the developing bolls. In Mississippi, Leffler and Tubertini[40] sampled cotton bolls from 7 days after flowering to maturity and found that boll K during the sampling period increased from 0.17 to 1.1 mg/boll[39]. Approximately two-thirds of the boll K was in the burs. Leffler and Tubertini[40] concluded that K in the burs serves as a reserve for the developing seed and fiber.

A general comparison of corn and small grain (wheat) crops with soybean and cotton shows a distinct difference in K accumulation that could have implications regarding nutrient management. As noted previously, most K is accumulated prior to silking in corn and prior to anthesis in small grains, whereas in soybean and cotton a majority of K is accumulated after the initiation of flowering. Since K is taken up by corn in such a short period during vegetative growth, Welch and Flannery[60] concluded that producers need to ensure that adequate available K is in the soil early in the growing season. Welch and Flannery[60] concluded that corrective applications of K after emergence would be difficult. This argument should also apply to small grains. However, for soybean and cotton corrective applications of K after plant emergence might be effective since K is taken up throughout pod and boll development, respectively. The high demand of the bolls for K and the amount of K that is accumulated after flowering have recently led to re-

search efforts to investigate the potential for correcting late-season K deficiencies in cotton by foliar feeding of K[50] and by in-row deep placement of K[48]. Later use by soybean and cotton also suggests that sidedressing K might be of value, particularly on sandy soils with the potential for leaching of K.

Potassium Concentrations

Potassium concentrations in vegetative tissues tend to decrease as the season progresses (**Figure 4**). This decrease in concentration reflects a dilution effect as total dry matter production exceeds the uptake of plant nutrients. Seed K concentrations, in comparison, typically stay relatively constant or increase slightly with time. As illustrated in **Figure 4**, K concentrations in the bur fraction of cotton bolls typically increase with time. Since K concentrations in various plant tissues vary as a function of time, the data in **Figure 4** demonstrate the importance of sampling the correct type of plant tissue at the appropriate time in order to use tissue analysis to evaluate K nutritional status. Critical nutrient concentrations (the concentration of a nutrient in a plant corresponding to 90 percent of maximum yield) are typically developed for a specific type of tissue (i.e. corn ear leaf, flag leaf on wheat) that is collected at a specified growth stage[2].

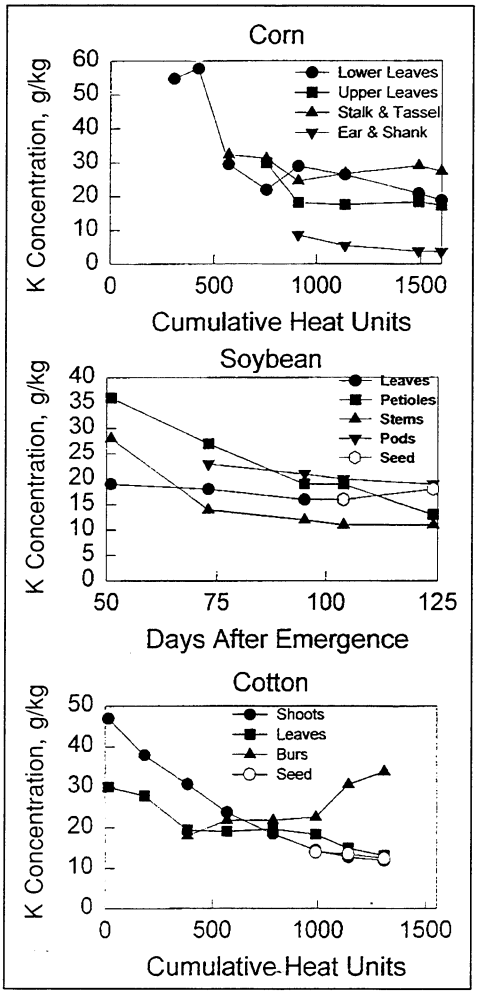

Figure 4. Concentrations of K in various plant parts of corn[36], soybean[25] and cotton[47] at various times throughout the growing season.

Soil Management and Plant Uptake of Potassium

In the previous sections, K uptake throughout the growing season has been discussed primarily in terms of differences between some of the more common major crops. Under actual production conditions, total K uptake can be affected by many management factors, including the fertility level of the soil, cultivar selection, tillage system, soil compaction, and others. In this section, selected examples will be used to illustrate some of the potential factors that may influence the uptake of K.

Fertility

Potassium uptake by any crop is affected by the level of available soil K and the rate of applied fertilizer K. Plant roots readily absorb K, and if abundant levels of available K are present in the soil, it is subject to luxury consumption. Some examples of how K fertilization impacts plant uptake of K are illustrated in **Figure 5**. In these tests, uptake of soil K by alfalfa and cotton increased with increasing rates of fertilizer K, but yields did not continue to increase economically beyond a certain optimum level. The data in **Figure 5** illustrate the challenge that producers have faced since the beginning of the use of fertilizers and also the challenge of all soil testing programs. That is, what level of fertilizer K is needed to produce economical and sustainable yields? As an example, Kerby and Adams[39] reviewed the literature on K fertility of cotton and concluded that optimum yields could be achieved if 0.1 to 0.13 kg of K is accumulated for every kg of lint. They associated higher levels with luxury consumption. Potassium fertility level can also affect the distribution of K within the plant. Cassman et al.[11] reported that cotton plants sampled at late fruiting contained 85 and 77 percent of the total plant K in fruiting structures on a soil receiving 0 and 480 kg K/ha, respectively. Coale and Grove[12] reported that for soybean, seed K accounted for 64 and 38 percent of the total plant K for low and high K fertility regimes, respectively. Potassium uptake is affected by the level of other nutrients. For example, total K uptake has been reported to increase with increasing rates of fertilizer N[17, 20, 34, 51].

Cultivar Effects on K Uptake

In some cases K uptake is dependent on crop variety or cultivar. Genotypic differences can be related to absorption efficiency of root systems as well as yield potentials. Barber and Mackay[5] compared K uptake of two corn genotypes under field conditions in Indiana. The genotype 'B73xMo17' took up an average of 1.34 times as much K as the genotype 'Pioneer 3732' at 75 days after planting (**Figure 6**). They concluded that the differences in

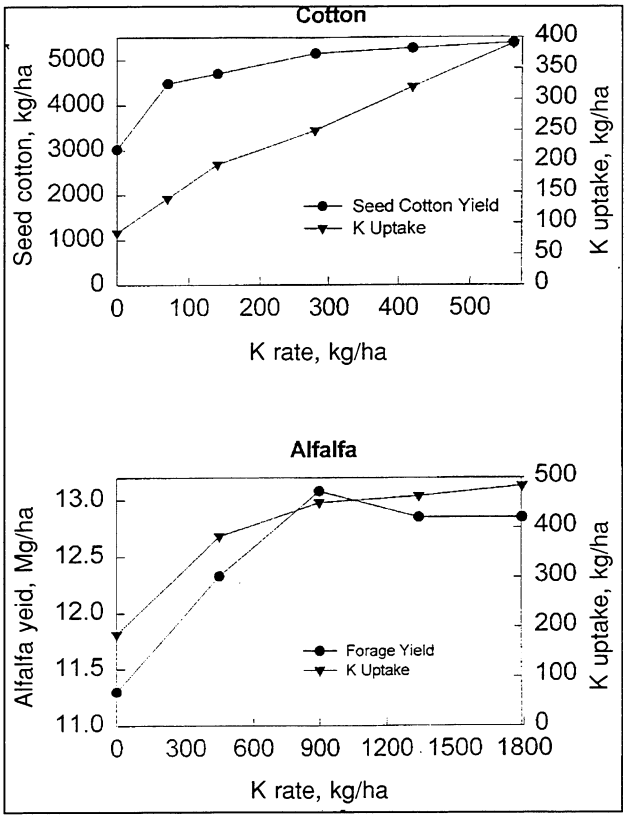

Figure 5. Potassium uptake and yield of seedcotton[9] and alfalfa forage[53] as affected by rates of fertilizer K.

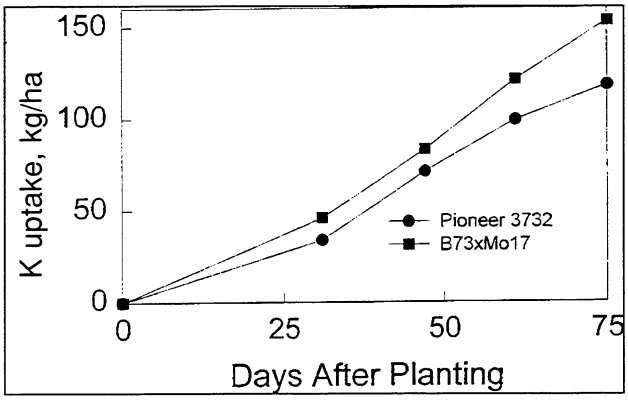

Figure 6. Plant K accumulation by corn cultivars 'Pioneer 3732' and 'B73xMo17' at various times after planting[5].

K uptake were due to a higher root growth rate and higher root surface area in 'B73xMo17' genotype. Hanway and Weber[23] compared eight soybean cultivars and reported total K uptake ranging from 78 to 103 kg/ha. Differences in total K uptake were associated primarily with yield potential. Another example of genotypic differences is work conducted with wheat in Virginia [Alley and Brann[1];as cited in Beaton and Sekhon[8]]. In this test, four winter wheat cultivars accumulated from 134 to 212 kg K/ha. In many situations, higher K uptake can be associated with higher yields, but in this test higher K uptake did not always correspond to the highest yield. Cassman et al.[11] demonstrated differences in K uptake in some Acala cotton cultivars. They grew two Acala cultivars on a low K soil. Under optimum fertility levels the cultivars produced the same lint yield, but without fertilization the K-use-efficient cultivar produced an average of 32 percent higher yield and 35 percent higher total plant K as compared to the inefficient cultivar. Bhatt and Appukuttan[10] evaluated nutrient accumulation by a bushy and a short branch cotton cultivar. The two cultivars produced the same cotton yield, but the short branch cultivar took up considerably less K.

There are also many examples where cultivar differences in terms of K uptake were minimal. Mullins and Burmester[47] compared K uptake by four modern cotton cultivars. During three site years of data, there were no differences in total K uptake or K uptake within a given plant part. More recently, Unruh and Silvertooth[59] compared an upland cotton cultivar 'Deltapine 90' with a Pima cultivar 'S-6' in Arizona and found no differences among the cultivars for total K accumulation. Karlen et al.[35] investigated K accumulation by 3 (1978) or 4 (1979) determinate soybean cultivars from maturity group VI, VII or VIII. They showed that there were generally no differences among the cultivars. However, at one sampling, the highest yielding cultivar had a higher petiole K level as compared to two of the other cultivars. Diebert and Utter[14] also looked at K uptake by an early and a late maturity soybean cultivar under three tillage systems. Total K uptake was the same among the two cultivars and the authors concluded that K uptake was determined by the effects of climate and tillage. The sometimes overriding effects of growing conditions on K uptake can be demonstrated by comparing the results of Mullins and Burmester[47] and Unruh and Silvertooth[58, 59]. Cotton in the study of Mullins and Burmester[47] was grown in Alabama without irrigation. The studies of Unruh and Silvertooth[58, 59] were conducted in Arizona under irrigation. Both sites were considered high in K fertility and both studies included the cultivar 'Deltapine 90'. Deltapine 90 in the study of Mullins and Burmester[47] accumulated a total of 106 kg K/ha with an average lint yield of 939 kg/ha whereas under more optimum growing conditions in the studies of Unruh and Silvertooth[58, 59] this variety accumulated 254 kg K/ha with an average lint yield of 1328 kg/ha.

Hocking[33] studied K uptake by a semi-dwarf spring wheat cultivar. He compared the uptake of K observed in his study with the results of previous research and concluded that plant breeding efforts to generate current semi-dwarf wheat cultivars has had little effect on the nutrient distribution among plant parts. This may be true for other crops as well. A comparison of the seasonal uptake of K by corn (expressed as a percentage of the total yield and uptake) as reported by Sayre[55] and Karlen et al.[38] are presented in **Figure 7**. Corn grain yields

and K uptake in the study of Karlen et al.[38] were 1.7 and 2.6 times higher, respectively, as compared to the test of Sayre[55]. Although the two studies were conducted 42 years apart (i.e. four decades of plant breeding and improved management/fertilization practices) and there were large differences in K uptake and yield, corn grown in the two studies had very similar seasonal accumulation patterns for both dry matter and K.

However, this relationship may not be true for cotton cultivars. Plant breeding efforts have resulted in higher yielding, faster maturing cultivars that partition a greater proportion of the dry matter into the fruit[45]. A comparison of K uptake data of Olson and Bledsoe[49] with those of Mullins and Burmester[47] shows that modern cultivars accumulated K during a much shorter time period as compared to the older cultivar. Some researchers believe that accumulating the seasonal K requirement in a shorter time period may be placing more stress on the cotton root system, and may be one of the reasons for more frequent occurrences of late season K deficiency in some locations.

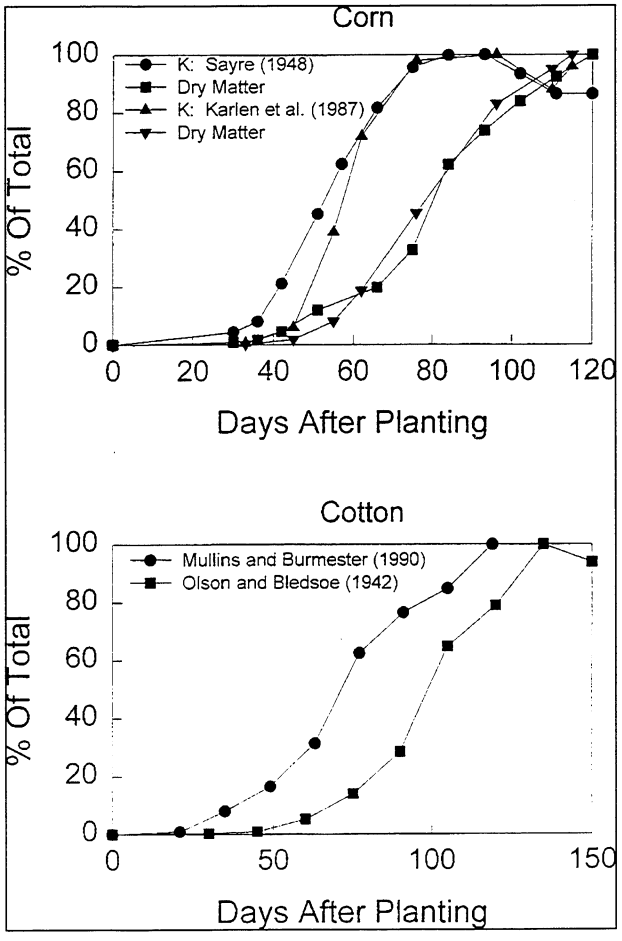

Figure 7. A comparison of the percentages of maximum K and dry matter accumulated during the growing season by corn[38,55] and K accumulation by cotton[47,49] grown in 1940 versus the mid-1980s.

Effects of Tillage, Compaction and Irrigation

The adoption of conservation tillage systems has resulted in chemical properties in the surface root zone that are different than in conventional tillage. In conventional tillage, surface applied K fertilizer is incorporated into the soil resulting in a relatively uniform level of plant available K throughout the zone of tillage (plow layer). Conservation tillage systems, in comparison, have limited, if any, incorporation of surface applied K or plant residues into the soil. This lack of disturbance results in a stratification of K, organic matter, and other nutrients in the surface soil[13, 52]. A concern with conservation tillage systems is the potential effects that stratification of K and other nutrients may have on nutrient uptake efficiency. Hargrove[28] grew irrigated corn on a Coastal Plain Piedmont (Cecil) soil in Georgia and evaluated grain yields and K uptake as affected by four tillage systems: 1) conventional tillage, 2) no-tillage, 3) reduced-tillage with shallow (10-15 cm) in-row chisel, and 4) reduced-tillage with deep (30 to 45 cm) in-row chisel. In this test (**Table 3**) conservation-tillage systems (with and without in-row chisel) resulted in larger plants and greater grain yields. Conservation-tillage treatments resulted in higher K uptake as compared to conventional tillage despite stratification of extractable K at the soil surface. Triplett and Van Doren[57] also reported K uptake by no-tillage corn equal to or greater than conventional tillage corn. Mackay et al.[41] looked at corn grain yields and K uptake in a site with conventional, no-tillage and ridge tillage systems for 9 years. The no-tillage treatment in this test had surface exchangeable K levels that were 2.4 times higher than the conventional tillage treatment. Potassium uptake by corn up to 64 days after planting was slightly higher in the conventional tillage treatment as compared to the conservation tillage treatments, but at 77 days after planting (midsilk) there were no differences among treatments (**Figure 8**). Using a mechanistic model, Mackay et al.[41] predicted that 47 to 52 percent of the calculated K uptake at 30 to 77 days after planting came from the upper 75 mm of soil as compared to 26 percent in the conventional tillage system. In another test involving more long term tillage treatments, Deibert and Utter[14] evaluated K uptake by soybean as affected by tillage treatments initiated 7 years prior to conducting their study. Treatments included both conventional and reduced tillage (fall sweep, intertill, and no-tillage) systems. During this three year study total K uptake by soybeans at pod fill (R5) and by the mature seed (R8) was not affected by tillage treatments.

Table 3. **Effect of tillage system on corn grain yield and total K accumulation by corn[27].**

Tillage treatment	Grain yield† Mg/ha	K uptake kg/ha
Conventional	8.61 a	249 b
No-Tillage	9.29 a	286 a
No-Tillage, deep in-row chisel	9.24 a	315 a

†Means in a column followed by the same letter are not significantly different at the 5 percent level of probability.

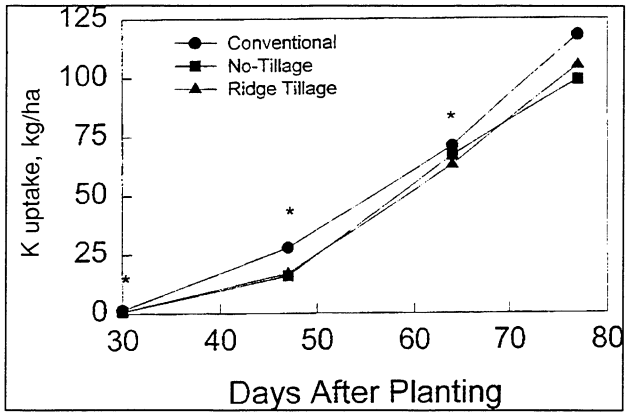

Figure 8. Total K accumulation by corn grown under conventional, no-tillage and ridge tillage systems in Indiana[41]. '*', indicates significant differences among tillage treatments at the 0.05 level of probability.

Another factor potentially affecting K uptake is soil compaction resulting from traffic. Dolan et al.[15] applied interrow, surface (0 or 4.5 Mg axle load) and subsoil (0, 9, and 18 Mg axle load) compaction treatments to a Webster (Typic Haplaquolls) soil and measured K uptake by corn at the 75 percent vegetative-tassel (VT) stage. When averaged over years (1982-1986) subsoil and surface soil compaction decreased K uptake between 8 and 21 kg/ha (7 to 19 percent). The authors concluded that surface soil compaction decreased dry matter production and also K uptake to a greater extent in the absence of subsoil compaction.

The primary supply mechanism for K in soil is diffusion[4]. Soil moisture can affect the supply of K to a root system by affecting the cross-sectional area of diffusion and the magnitude of the diffusion coefficient[4]. Thus, irrigation under water stressed conditions would be expected to increase K uptake by a crop. Karlen et al.[35] evaluated K uptake and yield of soybean as affected by irrigation. They reported that K uptake under stressed (nonirrigated) conditions fluctuated as a function of water availability. Under irrigated conditions K uptake was relatively constant until maturity. During a year of low rainfall, irrigation increased K uptake and yield by 37 and 62 percent, respectively. The authors concluded that increased K uptake resulted from increased dry matter production since irrigation did not affect the concentration of K in the corn tissue.

Summary

Potassium is an essential nutrient and is the most abundant cation in plant tissues. It is absorbed in large amounts by most crops and its total accumulation may be as much as 400 kg/ha or more under favorable growing conditions. With the exception of forage crops, a large proportion of the accumulated K is returned to the soil as plant residues after harvest. Corn and small grains, when harvested for grain, and seed cotton typically remove about 20 percent of the total plant K. Soybean seed, however, contain about 50 to 60 percent of the total plant K.

A majority of the total seasonal uptake of K occurs during a small proportion of the plant's growing cycle (typically 2 to 6 weeks). During this rapid uptake period average daily K uptake rates may range from 2 to > 20 kg/ha/d, with the actual peak rate depending on the crop and growing conditions. In corn and small grains the peak or critical uptake period for K occurs during the vegetative stage of growth. For these crops most of the seasonal uptake of K occurs before pollination in corn and anthesis in small grains. In soybean and cotton, however, the critical uptake period occurs during the start of flowering. Soybean, for example, accumulates about two-thirds of its total seasonal K uptake after flowering. A producer needs to be aware of the critical uptake period for a given crop and take appropriate steps in the fertilizer program to ensure that adequate K is available during the rapid uptake period.

Potassium concentrations decease with time in vegetative plant tissues. Seed K concentrations remain relatively constant with time. Tissue analysis can be a valuable tool in identifying K deficient plants, but the growth stage and the tissue sampled must be considered in the interpretation of results.

Seasonal K uptake is highly dependent on the crop, but several other factors potentially affect K uptake. These include cultivar, K fertilizer rate, levels of other nutrients, tillage system, soil mineralogy, soil compaction, environmental conditions, and irrigation. In the future, as new cultivars are developed and improved management practices are implemented (i.e. precision farming, etc.), efforts will need to be made to determine how these factors affect K uptake under a given set of cropping conditions. These efforts should help a producer to ensure that K is not limiting crop yields.

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Chapter 16:
Potassium Nutrition for High Crop Yields

U. Kafkafi[1] and G.H. Xu[2]

[1]*Department of Field Crops, Vegetables and Genetics, Faculty of Ariculture, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76100 Israel*

[2]*College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095 P.R.China*

Introduction

Leaf analysis has been widely used in an attempt to define the nutritional status of plants[36] for fertilizer recommendations[11, 12, 36, 55, 61]. Tissue K concentration is usually defined as low (deficient), adequate (sufficient), or high (excessive) for a particular plant organ, sometimes at an identifiable plant developmental stage. The definition of low and high levels of K concentration varies between plants as shown in **Table 1**. In avocado (*Persea americana* Mill.) the K ranges for leaves vary from <3.5 to 7.4 (low); to 7.5 to 20.0 (sufficient) and 21.0 to 30.0 (high) mg/g on a dry weight basis[36]. Potassium concentration varies widely in the various plant organs. Fleshy tissues like fruits and leaves at their early growth stage contain high levels of K. Potassium uptake usually precedes dry matter production[22]. Fleshy fruits, including cotton bolls (*Gossypium* sp.)[10, 30, 63], accumulate K to concentrations above 40 mg/g of the dry weight[12]. Grain crops take up soil K generally before grain filling or flowering state. The K found in ears totally depends on retranslocation from the other plant organs[2, 33, 39]. Flowers, developing fruits, and tubers serve as sinks for K, mobilized from the leaves. When K levels in the plant are low, K deficiency in leaves and a reduction of their photosynthetic activity[47, 50] are found during the fruit growing period. The K concentration in the leaves decreases to low levels as the fruiting season progresses. In wheat[28] and soybean[33, 35], little variation exists in the final K concentration in the grain of these crops despite differences in yield, even where significant differences among cultivars are found in levels of K at the vegetative stage. The higher the fruit or grain load the more severe is the depletion of K from the leaves, stems and, in some cases, the roots[13]. If the rate of K demand by the fruits or tubers is greater than the K uptake from the soil, all other organs may contribute K to the developing reproductive organs. As a result, K concentrations decline in the leaves during fruiting or grain filling. When the fruits are removed, increase in leaf K is found in

Table 1. Foliar concentrations of K of various fruits and vegetables (from Jones et al., 1991) and other annual crops (from Fageria et al., 1991).

	K level, mg/g				K level, mg/g		
Plant	Deficient	Adequate	High	Plant	Deficient	Adequate	High
Fruits				**Fruits**			
Almond	10	14	14	Papaya	28	33	55
Apple	10	15	20	Peach	10	20	30
Apricot	20	25	30	Pear	8	10	20
Avocado	3.5	7.5	20	Pecan	8	12	25
Banana	30	38	50	Pineapple	20	22	30
Blueberry				Plum, Prune	10	16	30
Highbush	3	5	9	Raspberry	10	15	30
Rabbiteye	3.5	6	9	Tomato	10	29	50
Cashew	7.2	8.9	14.4	Walnut	9	12	30
Cherry							
Sour	12	16	21	**Vegetables**			
Sweet	15	25	30	Garlic	30	39	48
Citrus *	7	7-11	12-17	Watermelon	30	35	45
Cranberry	4	8	8				
Currant, Black	8	14	17	**Annual crops***			
Fig	7	9	10	Barley	20-28	23-41	28-41
Grape	10	13	14	Common bean		15-35	
Grapefruit				Corn		20-25	
Non Friuting	6	8	22	Cowpea		20-25	
Fruiting	6	8	22	Potato		35-65	
Hazelnut	4	7	24	Rice		29-35	
Lemon	7	10	20	Sorghum	15	15-20	20-30
Macadamia	4	5	10	Soybean	12	17-25	26-28
Mandarin	4.7	9	11	Sugar beets	10		
Oil palm	16	17	19	Sugar cane		12-20	
Orange	4	7	11	Wheat	20-26	23-36	32-36

* From "Growth and Mineral Nutrition of Field Crops"[22].

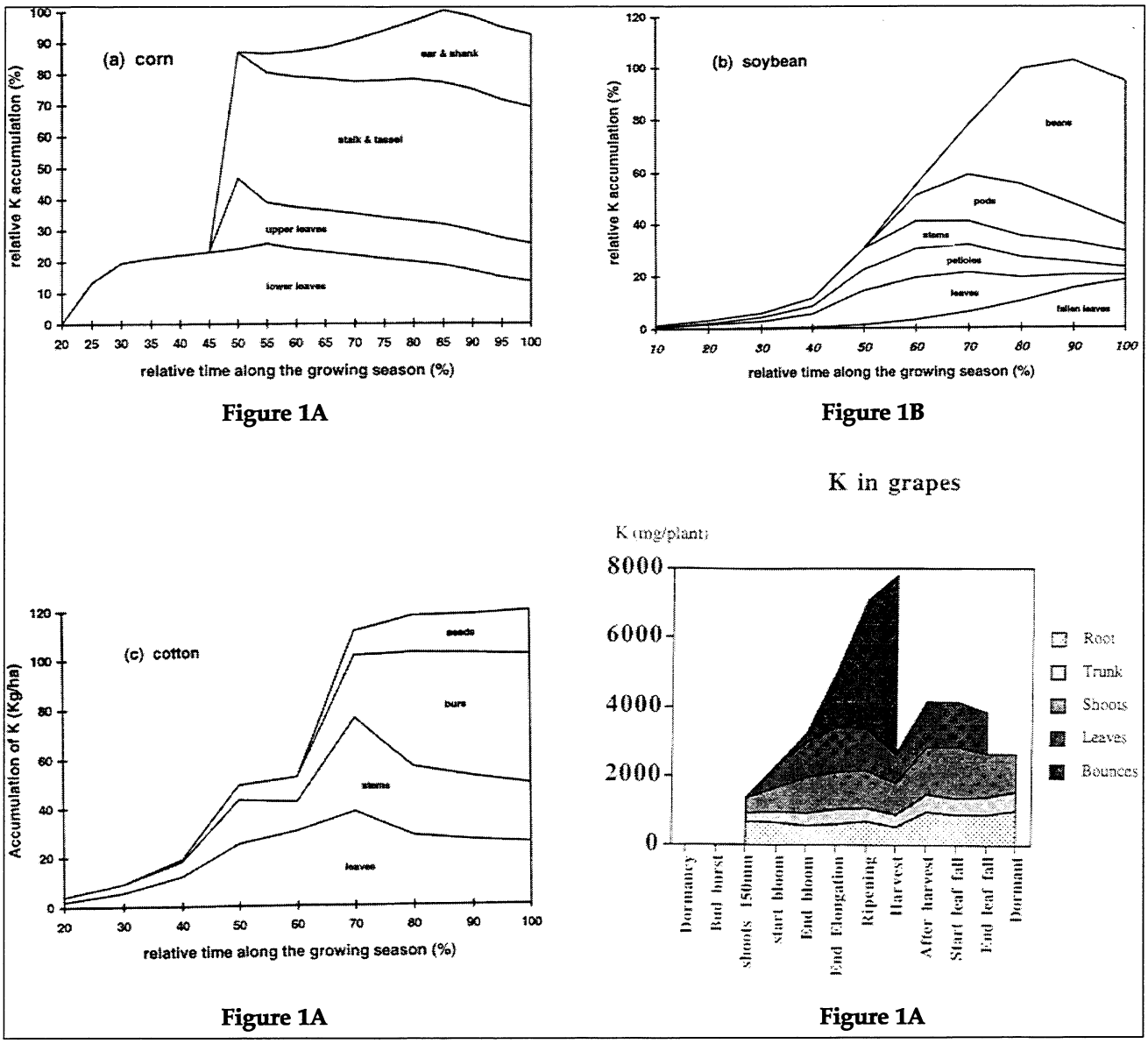

Figure 1A

Figure 1B

Figure 1A

Figure 1A

Figure 1. **Total relative quantity and distribution of K during growing season or one cycle. (Resources: corn: Karlen et al., 1988; soybean: Hanway and Johnson, 1985; cotton: Kerby and Adams, 1985; and grapes: Conardie, 1981).**

fruit trees[4, 10, 13, 63]. The reproductive organs demand for K may be so high[8] that K deficiency symptoms may develop in the leaves as commonly observed in potato and cotton. Leaf K concentration varies with time during the season. High concentrations of K may be necessary to achieve high yields and the desired large fruit size demanded by the markets.

Distribution of Potassium in the Plant

The amount of K removed from a unit soil area varies with the particular crop. The K accumulation rate and its distribution among various organs are well documented for some annual crops as shown in **Figure 1A-C**. Annual crops, such as soybean[33], cotton[30], corn[39], wheat, and rice[3] dramatically decreased K uptake from the soil after flowering or from the grain filling stage. Data on distribution of K in perennial crops are much more limited. Some relevant data for grapes (*Vitis* sp.) have been reported by Conardie[13]. The total quantity and distribution of K in grape vines during a one year cycle are presented in **Figure 1D**. Potassium is continuously taken up by the plant from just after bud burst in August until one month after harvest in February. The bud burst demand for K is supplied from stored K in the roots and the trunk. Potassium uptake by the roots then satisfies the K demand during the growing period. Most of the K is concentrated in the developing fruit. During the last month before harvest, the rate of total K uptake cannot keep up with the demand of the fruit. The fruit K requirement is then satisfied from stored K in the leaves and shoots. The phenomenon of K retranslocation is very similar to that found at the final growth stages of annual grain crops, cotton[51] (**Figure 1A-C**), potato[52] and sugar beet[17]. This implies that when the demand for K

by the developing fruit cannot be satisfied by uptake, K is removed mainly from the leaves and the shoots. As a result, the amount of K in the leaf of grape vine at harvest (**Figure 1D**) was 35 percent lower than one month earlier. The moment the fruit is harvested, K increases in all plant parts. A significant portion of post-harvest absorbed K is retained in the permanent parts of the vine.

Movement of K from the leaves to a developing sink by phloem transport is found in all plants and organs[49] and has been demonstrated in potatoes (*Solanum tuberosum* L.)[15], tomatoes (*Lycopersicon esculentum* Mill.)[32], apples[31], prunes[45], and peaches[54]. In potatoes, a flow of carbohydrates containing 2 mg K/g dry matter is constantly moving to the developing tuber. Increasing the K content in potato leaves from 23 to 30 mg/g has been shown to increase tuber yields and lead to an increase in the resistance of the foliage to frost damage[27].

Potassium is required for the proper functioning of a number of enzyme systems at a concentration of about 50 to 100 mmol/L (on a fresh weight basis)[5, 47]. However, K is found in higher concentrations, on the order of 200-300 mmol/L, in K sufficient plants. Leigh and Wyn Jones[43] explained this higher K concentration required for growth with the distribution and functions of K in plant cells. They employed a simple two-compartment K model: vacuole and cytoplasm. Potassium stored in the vacuoles acts as a reserve pool which aids in keeping the active cytoplasmic K at a near constant level. Potassium can be drawn from the vacuolar pool to satisfy new demands such as those exerted by the fruit, but cytoplasmic K levels must be left intact. At a low K level in the plant, utilization of cytoplasmic K by the developing new sinks can have adverse effects on K-mediated metabolic processes in the cytoplasm.

It is obvious that when foliar K deficiency symptoms develop as a result of fruiting, the fruit has drawn on cytoplasmic K. At what level of leaf K fruit sinks begin to deplete cytoplasmic K needs to be determined for each crop. Even if this cytoplasmic depletion occurs to the slightest degree, the depletion may have an important overall effect on the plant metabolism and yield[5].

Plant Potassium Requirement

Potassium demand varies throughout the growing cycle. Perennial fruit plants take up K throughout the growing season, whereas annuals such as cereals and sunflower reach the peak of K content around flowering with a decline towards maturity[29]. Insufficient K supply reduces the total dry matter production and distribution within crops, but the reproduction organs are the most drastically affected, hence the particular importance of K in fruit, tubers and grain crops. Low K supply to banana reduced bunch weight by 80 percent, but did not affect root growth[42]. The K requirement for optimal plant growth ranges from 20 to 50 mg/g of the dry weight of the vegetative organs, fleshy fruits, and tubers[22, 47]. However, the current recommendations of K levels in plants from various sources for fruit production are rather low. Most of the recommendations are still based on data that were published more than 50 years ago[36]. The data for apples (*Malus domestica* Borkh.)[11] illustrate the change in recommendations over time for intermediate foliar K concentrations: In 1931, the recommendation was 4.2-16.5 mg K/g of leaf dry weight; by 1948 this changed to 12-37 mg K/g. For blueberries (*Vaccinium* sp.), the recommended sufficiency K range was 1.0-1.5 mg K/g of leaf dry matter in 1951 despite the fact that a shortage of K was associated with terminal buds growing point abortion[9].

Abundant information exists on the concentration of K in crop leaves, but only limited information is available on leaf K in relation to time during the development of reproductive organs, especially towards final yields in fruit trees. The effect of fruiting or fruit load on foliar K concentrations is demonstrated in **Figure 2**. McClung and Lott[48] reported that the biggest difference among peach leaf nutrients in the presence of fruit was in the concentration or total quantity of K in the leaves. Leaves of fruiting trees contained only about 50 percent of the total K present in non-fruiting trees (**Figure 2C**). This effect of fruiting on foliar K content was not shown with foliar nitrogen (N), calcium (Ca), magnesium (Mg), or phosphorus (P). After flowering, leaf K of peach steadily decreased in fruiting trees while non-bearing trees exhibited a constant leaf K throughout the growing season. Similar results are also shown in **Figure 2** with apple[31], walnut[19], litchi[4] and cotton[2]. Similar behavior was observed with prune[44]. Leaf K concentration of walnut decreased from 18.1 to 22.3 mg K/g in young growing leaves to 8.6 to 18.5 mg K/g in the mature leaves and 8.0 mg K/g in old leaves at the end of the life cycle. The estimated reduction for K accumulation in leaves was 48 percent from the younger mature leaves to fruit during the fruit developing stage[19]. Even though the total yield of litchi is low, the demand for K to the developing fruit from accumulated foliar K reserves is high. Tomato also exhibits a wide range of foliar K concentrations, heavy yields causing leaf K to decline from flowering until harvest. After harvest, foliar K content increases[49], although this increase is dependent upon the soil K supply.

In grain crops, the K content of the grain as a percentage of total K in the above ground parts of cereals is low; about 16.3 to 33.9 percent for corn[64], and 18.0 to 24.0 percent for most small grain crops[6]. Potassium content in grain maintains a relatively uniform level and is less influenced by K fertilizer than that in the straw. Furthermore, incorporation of labeled K^+ into the wheat grain was not affected by the previous (endogenous) K content of the plant[28]. Hybrid rice grain K content as a per-

centage of total K in the plant increased from 20 to 24.6 percent[53]. Much higher percentages of K in reproductive organs to total whole plant K are found in fleshy storage tissues: about 60 to 70 percent in potato tubers[52], 35 to 56 percent K in the ears of corn[14, 39]; 60 to 68 percent in bolls of cotton[2, 40, 51, 63] and about 25 to 35 percent in roots of sugar beet[17]. This part of K is partly drawn from stems and leaves as shown in **Figure 1**.

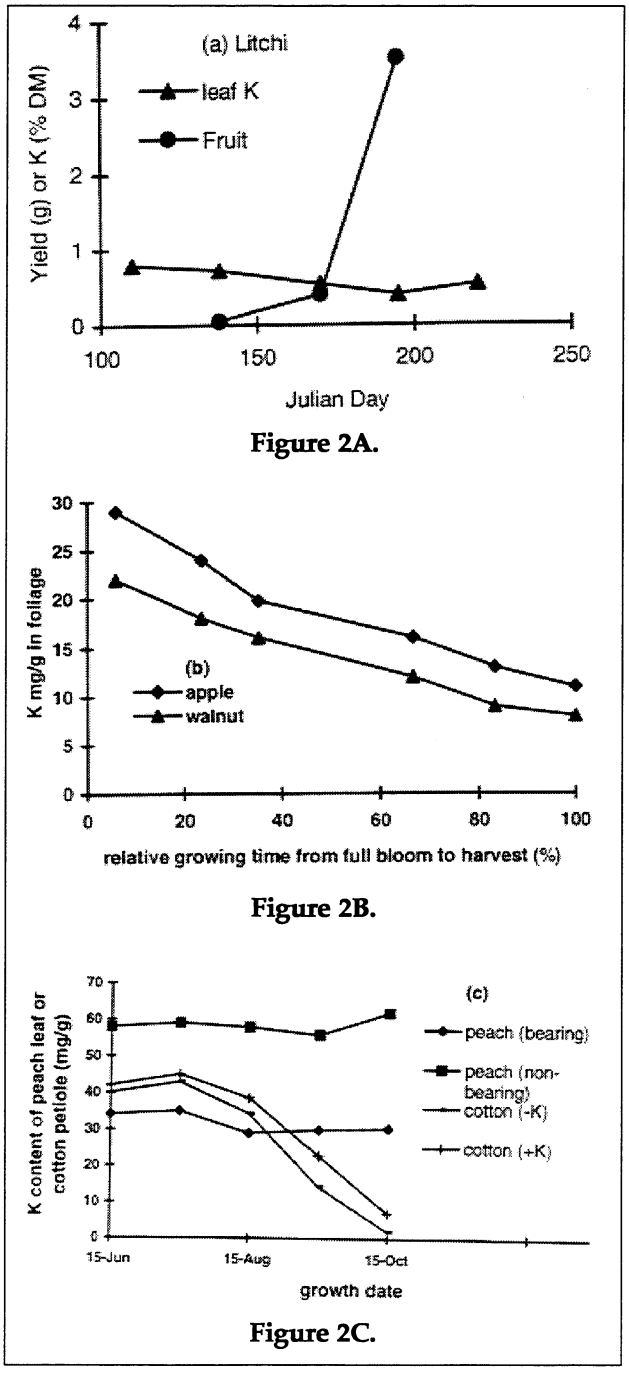

Figure 2. Variation of K percent at different reproductive states. (Resources: litchi: Bar and Glusman, 1991; apple: Cummings, 1985; walnut: Drossopoulos, 1996; peach: McClung and Lott, 1956; and cotton: Weir et al., 1986).

Potassium Reserve Pools

The question must be raised: What is the total level of K in readily available reserve pools in the leaves and stems that must be present in the plant at the initiation of flowering or fruiting to secure high and quality yield? The answer to this question is crucial. During fruit growth or grain filling the main sink for carbohydrates is the developing fruit or grain. The primary organ that suffers from depleted carbohydrate supply at that time is the root. The root activities of corn, wheat and cucumber fall at fruiting time to the lowest level of the growing season[65]. As a result, K uptake from soil declines, and the leaves become the main source of K for the developing fruit. Some cereals even exude K from their roots during grain filling[3].

Values of critical K leaf content for field crops vary between 12 mg/g and 20 mg/g (**Table 1**) at about shooting or flowering stage[22]. The critical value depends on crop, variety, growth stage, sample position (organ) and climate. Significant positive relation between K concentration or K accumulation of leaves and stems at flowering stage and final grain or fruit yield are presented in **Figure 3**.

When the K level in the leaves is reduced below a critical level, the leaves shed. Leaf shedding suppresses carbohydrate supply to the developing fruits. Young fruits require ample carbohydrate supply for fruit set. In cases such as litchi, avocado and citrus, the newly developing fruits shed prematurely, possibly because the supply of K that is essential to the phloem flow of carbohydrates from the leaves to the fruits becomes the limiting factor[47].

Rootstock is a primary factor affecting leaf K concentration of sour cherry. Fruit load and weather conditions are also two major factors provoking considerable seasonal fluctuations of leaf K content[34]. Potassium concentration in avocado leaf (**Figure 4**) declines with flower development from late February to March. The older leaves from the previous season are active until March and then, two weeks later, young leaves develop which are high in K. Development of flowers and new leaves occur simultaneously, both of which demand high amounts of K. These processes occur in March, when the soil is still cold and root uptake of K is limited. Therefore, both leaves and fruit must draw K from the trunk, root, and bark reserves. In cherry trees over a nine-year period, the average leaf K contents of two varieties of unfertilized trees were 7.0 mg/g and 12.1 mg/g, while the mean values on fertilized plots with annual K contents of 166 kg K/ha were 9.8 mg/g and 17.0 mg/g, respectively[34]. These values demonstrate the difficulty in obtaining a single, distinct number for leaf K concentration that will be correlated with fruit yield.

The importance of K reserves in the tree for growth and yield in the next season was demonstrated for apple by Edgerton[21]. Varying amounts

of K were supplied in the first growing season and then K was withheld from the trees during the following season. Trees with low K reserves (low K supplied in the first year) showed deficiency symptoms during the next season. Foliar K concentration values from the second season ranged from 2.4 to 33.5 mg/g. Visual leaf scorching symptoms due to K deficiency were not observed when the leaf contained more than 7.5 mg/g[50]. However, yield reductions were observed when foliar K concentrations were higher than those causing visible symptoms in the leaves. In apple, at 10 mg/g leaf K, leaves are free of visible symptoms, but the fruit does not color normally[24]. In pears, a response in fruit size was obtained when K concentrations in dry leaf matter[25] were between 7.0 and 10 mg/g. Deficiency symptoms were only observed at the 7.0 mg/g level.

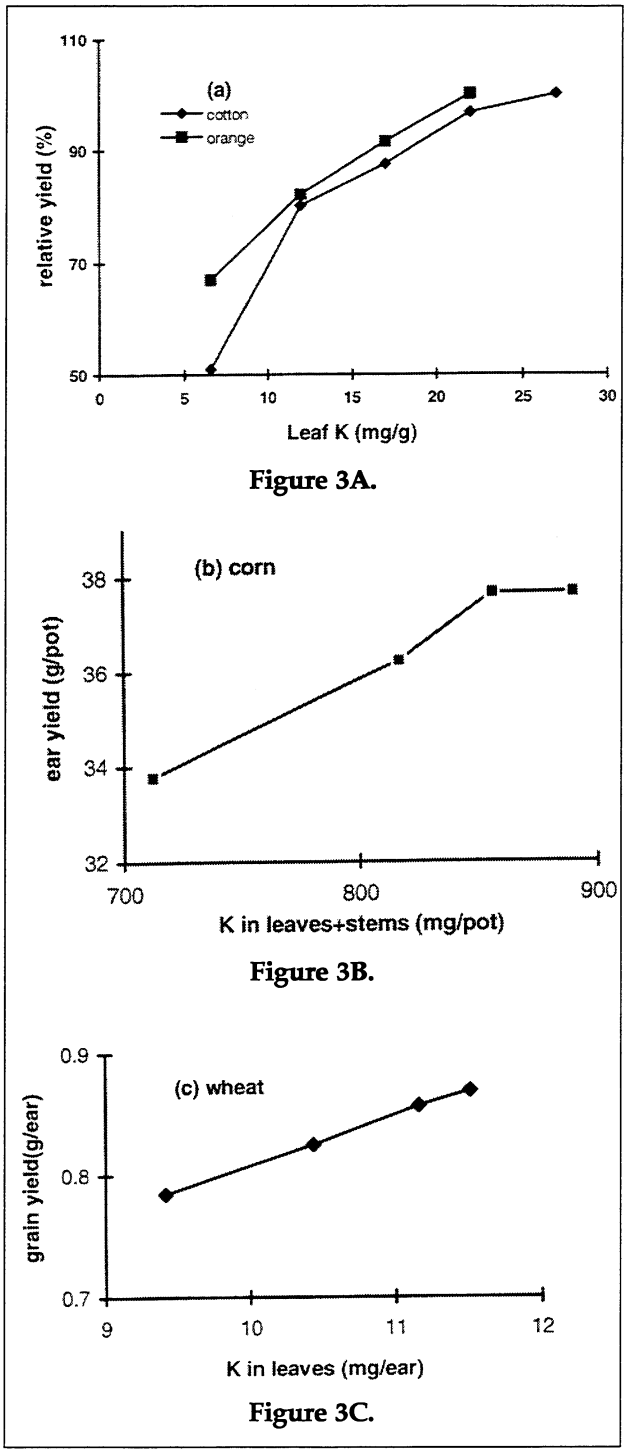

Figure 3. Relationship between fruit and seed yield and K content of leaves and stems. (Sources: cotton: Bennete et al., 1965; orange: Koo, 1985; corn and wheat: Xu

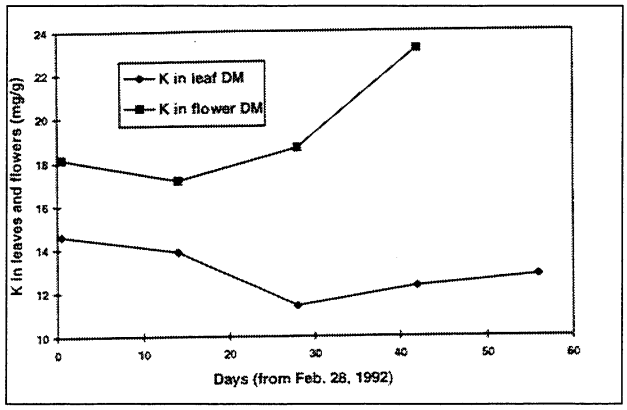

Figure 4. Relationship between K content of developing avocado flowers and leaf K status. (Resource: Bar and Glusman, 1991).

Similar effects of the fruit as a K sink on the content of K in the leaf have been documented in citrus by Smith[59]. However, the values of plant K before flowering are missing from his careful documentation, so the K level at which no hidden deficiency will develop later on in the season due to heavy fruit yields is not known.

The minimum level of K in the leaves that is needed to maintain the maximum flow of sugars to the developing fruit needs to be determined. This concentration would probably be a function of the number of fruits (sink size) and their rate of development. The leaf K concentration that can maintain continuous K transport to the flowers and the developing fruit as a function of time is an unknown value for most tree crops. It is expected that the required level for continuous K transport to the fruits must be higher than the presently recommended leaf levels of K if higher yields are to be obtained. When fertigation systems are available, it is possible to supply K at the time of fruit demand. However, we know very little of the functioning of the root system during fruit filling period. Therefore, it is imperative that a better understanding of the impact of the plant K reserve pools on K supply to the fruit be determined. High levels of K nutrition are not necessarily relegated to a 'luxury' range. Such an understanding becomes more important with the advent of high density tree plantings and a shift towards smaller tree stature and fewer leaves per unit of fruit.

The value of K application via drip irrigation on the K status of prunes has been recently reported

for fruited versus defruited trees at stage II of prune fruit development[62]. Fruited trees took up 90 g more K than defruited trees. The application of K in the drip system was capable of maintaining the K supply needed by the plant.

Actual Potassium Fertilizer Requirement

The beneficial effects of pre-winter N and P fertilizer application to avocado on the yield of the following summer and the N fertilizer application to sweet corn on grain yield are shown in **Table 2.** The increase in yields was associated with lower levels of K in the foliage. Such an increase in yields and a decrease in plant K status in avocado and corn must be considered when interpreting plant K status. Yield increase can be gained by a better understanding of plant K relationships. As a result, a secondary benefit may be realized where more efficient utilization of applied N could be obtained. Removal of higher amounts of N by the plant also has a potential to reduce groundwater pollution.

Table 2. Reduction in K content of avocado and corn leaves with yield increase due to fertilization.

Treatment	Yield kg/tree	N	P	K
		--- In leaf dry weight (mg/g)---		
		-------- Avocado† --------		
Control	39.6	21.6	1.1	11.4
Fertilized	74.0	122.1	1.0	6.4
Fertilizers‡	g/pot	------ Sweet Corn§ ------		
$N_{100}K_{100}$	24.2 a	22.7	1.9	31.5
$N_{200}K_{100}$	28.2 b	27.4	1.8	28.9
$N_{200}K_{150}$	28.8 b	27.4	1.8	30.9

† Reuveni et al., 1990. Each tree received 30 g K.
‡ Fertilizers in mg/kg soil.
§ Chu Lin et al., 1989.

The most responsive crops to K fertilization are those with shallow root systems where the soil K supply is rapidly depleted with time. Scibisz and Mucha[57] noted that red currants (*Ribes pestraeum* Wulf.) receiving continuous fertilization with K produced higher yields and maintained greater plant vigor than plants receiving K in one dose. Due to their shallow root system, red currants respond to K additions more readily than deeper rooted trees such as apple, due to the potentially smaller soil volume utilized by the root system.

Recalculation of the data presented by Smith[59] demonstrates the range of K concentrations found in 'Valencia' orange (*Citrus sinensis* Osbeck) leaves from three fertilizer treatments (**Table 3**). When the K concentration on a dry weight basis was 8.3 mg/g, the solution in the leaf was 149 mmol/L. This K concentration is similar to that found in the cytoplasm[43] and indicates little or no K in the vacuole. Therefore, K reserves are very low, and when fruit growth commences, this shortage in vacuolar K reserves will be reflected in production of small fruit[59]. Increasing the Ca and Mg concentrations in the leaf at constant K concentration does not compensate for inadequate K reserves and fruit size remains small[58, 59]. This is not surprising since K comprises the major cation of fleshy fruits and vegetative tissues and plays a key role in turgor-regulated movement[47].

Table 3. Calculation of K concentrations in 'Valencia' orange leaves†.

Parameter leaf	K level		
	Low	Intermediate	High
K mg/g DM	8.3	11.2	18.1
g DM/100 g FW	41.3	40.1	38.2
g H_2O/leaf	58.7	59.9	61.8
K mmol/L leaf solution	149	192	286
g K/100 g FW	0.34	0.45	0.69
K mg/kg leaf solution	5840	7498	1,1188

† Using data from Smith et al., 1953.

When foliar K concentration of orange is 18.1 mg/g on a dry matter basis (**Table 3**) the K concentration in the leaf fluids is 286 mmol/L K. This level of K concentration is important to many functions at both the cellular and whole plant level. Frost hardiness is increased by high levels of K^1 as well as cold resistance of some fruit crops and flowers[38]. Stomata remain open at night at low K concentrations and close tightly when ample K is present in the leaf[47]. Since fruits grow in volume mainly during the night[24] preserving tree turgor during the night is important in securing large fruit size.

It is a puzzle why foliar K levels recommended by field advisors for fruits, such as blueberry, orange, and avocado, are still very low (**Table 1**) despite the available information that suggests the opposite. The concentration range of K in dry matter defined as 'normal' by various researchers for blueberry ranges from 1.3 to 18 mg/g[9]. Such huge variation in estimates needs reassessment. This reassessment should be based on an analysis of K distribution within the various plant organs at definitive physiological sampling times. Eck[20] recommended an optimum K sufficiency range between 4.5 and 5.5 mg/g for blueberry. However, these data were based on foliar samples taken one week after harvest. This is the period when foliar K is at a minimum and has little bearing on the true K status or requirement of the plant for maximum productivity. It is clear from the compiled data of Jones et al.[36] and Fageria et al.[22] (**Table 1**) that the upper limit of K considered sufficient for most fruit trees corresponds to the lowest levels suggested as a low K concentration for vegetables. It is possible that the low levels of K in leaves are responsible for the low yields obtained in citrus and avocado despite the huge number of flowers that these plants bear. Avocados have between 1 and 1.5 million flowers per tree. Each truss contains 150 to 250 flowers, weighs about 1.7 g and has a K concentration of 25 mg/g. These data suggest that the amount of K in the trusses of one plant may approach 210-310 grams just to satisfy the need of

the truss for K. Simple calculations such as this underline the importance of increasing the dosage of K for increasing fruit yields. If the Leigh and Wyn Jones[43] model is correct, the K level in the cytoplasm may be satisfied, and the fruit yield will still be low simply because the flow of K to the developing fruit becomes the limiting factor for a short period due to the insufficient level of mobile K in the vacuolar reserves. It seems reasonable that total K accumulated in mature leaves and stems at the start of the fruit setting or grain filling should not only meet the K need of developing fruits and seeds determined by final yield target, but also maintain the lowest K concentration for their biochemical functions in the leaf cytoplasm.

The mid-season of growth is usually the peak demand period for K of most crops and needs a very high supply rate of K from the soil to meet crop requirements. Sidedressing K at early ear emergence, jointing stage, or squaring in annual crops such as wheat, rice and cotton, respectively, often results in more effective yield increase than for preplant fertilizer[2, 3, 46]. As the root activity declines rapidly during the reproductive stage, supplement of K to crops through the leaves appears to be more effective than soil K application[60, 65]. Foliar application of K or soil K topdressing after 50 percent tasseling increased the grain yield of sweet corn by 51 percent and 23 percent, respectively, even though the K content of stems and leaves at harvest was still near 20 mg/g[60]. Suitable timing of foliar K fertilizer not only supplied some of the applied K to the sweet corn, but also stimulated K absorption from the soil by the plant. Foliar feeding of K increased or slowed the decline rate of root activity of corn, wheat and cucumber[64]. The fruit set of tomato sprayed with KH_2PO_4 did not cause a decrease in K content of the leaves and always maintained over 20 mg/g compared to 14 mg/g in the leaves of control treatment[37]. Therefore, the stimulating effects of foliar K application at the appropriate reproductive stage of a crop, an old idea, should be reconsidered as an effective means of ensuring K status of leaves in cases of severe deficiencies.

Summary

The developing reproductive organs of various crops demand a daily supply of large amounts of K to produce high quality fruit yields. The high demand for sugars in the developing reproductive organs reduces the sugar supply to the root and as a result K uptake declines, or stops completely. The ability of the perennial fruit trees to produce high fruit yields depends on the K reserves inside the plant before fruiting and the supply of K from the soil during fruiting. Annual grain, tuber, and fleshy fruit and vegetable crops as well as cotton translocate K from leaves and stems during reproductive organ development. Leaf analysis data over the growing season demonstrate that high yields cause leaf K to decline from flowering or jointing stages of field crops until harvest. After harvest, foliar K content of perennial fruit trees increases. Data from a number of fruit and grain crops lead to the suggestion that a much higher supply of K than currently applied is needed before the flowering or ear emergence periods. High fruit yields with concomitant alleviation of annual yield fluctuations in orchards and more satisfactory grain yields can be achieved through top dressing or foliar spray of K. It is suggested that total K accumulated in mature leaves and stems at the beginning of the fruit setting or grain filling period should be high enough to meet the need for K of the developing fruits and to maintain above-minimum K concentration needed for biochemical functions in the cytoplasm of leaf cells to allow continuous photosynthesis. The high K demand of crops and K removal from the leaves and stems during the reproductive developing stage warrant a reconsideration of the present low levels of leaf K content recommendations in orchard crops.

Synopsis of Future Research Imperatives

To ensure high quality and economic yields, the K status of plants during the growing season has to be studied. All major nutrients, as well as water conditions, in the soil and plant must be recorded at the time of sampling. Recording the leaf moisture content at sampling will enable the calculation of the real K concentrations in the cellular fluids and ascertain the validity of the Leigh and Wyn Jones[43] model. Reliance only on interpretation of K status on a dry matter basis for fruit producing crops is not expected to yield a true picture of plant K status. Hansen[31] has clearly shown that K in fruit trees accumulated in concert with the dry matter accumulation. Therefore, concentrations of K based on dry matter only will not give the differential data needed for critical quantitative evaluation of K requirements. The replenishment of K, especially to the woody parts of the plant after harvest, needs further study. The K stimulation of a high storage (sink) capacity formation in the grain through higher photosynthesis must be quantified. The effects of K nutrition on the level and balance of phytohormones is worth a detailed study[7]. The size of K sinks, such as flowers and fruit, on utilization of K reserves and the productivity the following season may assist in increasing the cold and drought tolerance of crops, reduce biennial bearing trends, and increase fruit size. Leaf diagnostic criteria during reproductive organ development should be interpreted in relation to the desired yield of the current season and the next season. The possibility of fertigation supplying K just before and at the time when fruit demand arises should be investigated for increasing fruit size, especially in fruit such as cherries, apricots, citrus, and plums where large fruit has a major economic advantage.

On-line leaf analysis techniques like chemical field tests, near infrared analysis and remote sensing spectra from canopies of field crops and or-

chards should be developed to enable the farmer to respond to shortages in real time and secure the yield for the current year. Sampling of a constant unit of surface area at a constant position of the leaf surface at a constant time during the day will allow a much better calibration of leaf sampling and allow comparisons between seasons. The moisture content as well as the dry matter content at the time of sampling should also be recorded for more accurate interpretation of plant K status.

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Chapter 17:
Modeling Soil-Plant Potassium Relations

Sylvie M. Brouder

Agronomy Department, Purdue University, West Lafayette, IN 47907-1150

Introduction

Models are essential, integral components of hypothesis development and testing. Mechanistic simulation models which mathematically characterize complex chemical, physical, and biological processes in soils and plants have made valuable contributions to our understanding of nutrient accumulation. In the simplest of terms, nutrient uptake can be characterized as a stepwise progression of events including (i) movement from the bulk soil to the root surface, (ii) transport across the plasma membrane of a root cell, (iii) radial symplastic movement toward the root xylem, and (iv) long distance transport to a nutrient sink in the plant via xylem and phloem[14, 50]. But, in reality, nutrient uptake results from numerous complex plant and soil processes interacting simultaneously in a variety of ways. The principle objective of mechanistic simulation modeling is to explore individual uptake factors and the processes that affect them, factors and processes that root/soil interactions make difficult to assess directly.

To date, much of the effort to develop a mechanistic description of the nutrient accumulation process has focused on potassium (K) and phosphorus (P). Not only are these essential elements required in large amounts by all plants, they are also multiphasic in soil. At a given time, the soil solution phase, the pool from which most root accumulation occurs, contains only a small portion of the total soil quantity of K and P. Soil K- or P-supplying power reflects both the ability of solid-phase ions to replenish the solution phase and the mobility of the ions in solution. But quantification of the plant- or bio-available pool also depends on the plant, especially the size of the root system, the nutrient uptake rate per unit of root, and the length of time a root remains active. For K and P, plant accumulation rate is often relatively rapid when compared to the rate of supply from the soil, and a depletion zone develops around the active root. The development of a depletion zone de-emphasizes the importance of mechanistically characterizing plant physiological processes as uptake will be determined mainly by soil supplying power. Thus, model assembly need only concentrate on the first step in the progression of accumulation events.

The objective of this chapter is to highlight the contributions made by mechanistic simulation modeling activities to the evolution of knowledge regarding K behavior at the root-soil interface. The discussion will focus on the approaches to model development and verification and relate some of the tangible advances in agricultural K management that trace to efforts to simulate K transport from the soil into the plant. An attempt is made to identify research imperatives for mechanistic modeling of soil-plant K relations, as well as to identify current applied research objectives that may be strengthened by adding a mechanistic modeling component to the experimental approach. This chapter does not address non-mechanistic, empirical or "hybrid", models, and it is not intended to provide a comprehensive review of the literature on modeling plant-soil nutrient dynamics as there are several excellent, recent reviews on nutrient uptake models and their scientific contributions[42, 66, 70].

Types of Models

In the study of soil-plant nutrient relations, models have generally been classified as either empirical or mechanistic[66, 70]. Empirical models describe observations without attempting a theoretical explanation. Such models are the foundation of whole-field fertilizer recommendation protocols derived from static soil tests. A simple correlation of relative yield to extractable nutrient quantities is used to predict the probability of a yield response to fertilizer addition[8, 49]. In contrast, mechanistic models are based on theory or established scientific principles that have been mathematically characterized. At a minimum, the mechanistic simulation of plant nutrient uptake requires mathematical description and integration of the soil and plant processes that affect nutrient movement from the bulk soil through the rhizosphere and across the plasmalemma of a root cell.

Mechanistic nutrient uptake models can be subdivided into two categories: one-dimensional (vertical) models and single-root models[70]. Vertical models characterize soil layer differences with depth in the soil profile, emphasizing gross changes in soil nutrient availability through space and time. These models have been applied to the study of water, nitrogen (N) and heavy metal accumulation[37, 63, 64]. Single-root models simulate radial flow of nutrients from a soil cylinder surrounding either single roots or multiple roots of a developing root system. These models, which use mass flow and diffusion to describe nutrient flux in the soil cylinder around a root, have been applied extensively to the study of plant-soil K dynamics (**Tables 1 and 2**). They typically assume a kinetic characterization of the nutrient transport across the root

Table 1. Simulation models that have been applied to the study of K uptake, the defining characteristics, and seminal references.

Model/Authors	Characteristics	Reference
Baldwin-Nye-Tinker (B-N-T)	Radial flux by mass flow and diffusion of single nutrient ion to randomlydistributed root system of time dependent density. Linear, non-mechanistic nutrient ion influx across the root surface.	Passioura and Frere, 1967 Nye and Marriot, 1969 Baldwin et al., 1972 Baldwin et al., 1973
Claassen-Barber	Nutrient ion soil flux as in B-N-T, with nonlinear Michaelis-Menten kineics for variable influx across the root surface. No inter-root competition for nutrient ions.	Brewster et al., 1976 Claassen and Barber, 1974, 1976
Cushman-Barber	Claassen-Barber model modified for root competition, time varying root influx/efflux, and age-dependent root parameters. Adapted for use with personal computers.	Cushman, 1979a, b; 1984a, b Barber and Cushman, 1981 Oates and Barber, 1987 Barber, 1995
COMP8	As in B-N-T/Cushman-Barber model with competition between two or more species of contrasting root length(s) and nutrient absorptive power(s). Variable soil buffer power.	Nye and Tinker, 1977 Smethurst and Comerford, 1993
Bouldin	Multiple cations and anions with focus on the maintenance of electrical neutrality of soil, soil solution, and plant. Interactions between exchangeable Ca, Mg, and K, but without consideration of interaction between ions during uptake. Non-mechanistic, linear influx functions.	Bouldin, 1989
Silberbush et al.	Multiple ions and salinity with chemical exchange of cations according to Capon isotherms. Mechanistic characterization of influx with interaction between ions during uptake, but no age or location effects on root uptake kinetics.	Silberbush et al., 1993 Yakirevich et al., 1994

cortical membrane, but they exclude nutrient distribution in the plant and feedback controls on uptake. As mentioned above, the oversimplification of the plant absorption process is thought to be less of a concern for K as soil supply characteristics are deemed more likely than root activity to limit uptake.

An additional classification term, "hybrid", has been used by Rengel[66] to describe models that fall between empirical and mechanistic with regard to the rigor of the theoretical basis. Rengel identifies several nutrient balance models that have a mechanistic description of only selected chemical, physical, and/or physiological properties, but rely on purely statistical descriptions for the bulk of the model relationships[10, 38, 41, 85]. It should be noted, however, that model classification is highly subjective, and most modeling activities are technically "hybrid" efforts. Indeed, at some level, all mechanistic models also contain structurally important empirical relationships, since authors of mechanistic simulation models make numerous assumptions during model assembly. The assumptions may reflect intentional simplifications designed to keep the models from becoming too cumbersome to verify or apply, or they may reflect component phenomena that are poorly understood. For poorly understood component phenomena, an empirical relationship has to be used to describe that model component until more information allows a functional substitution. Ultimately, we must remain cautious with our use of classification terms and the import we give them. The judicious use of mechanistic models should be grounded in the understanding that theories reflect scientific consensus, which may not be synonymous with scientific truth.

Approaches to Mechanistic Simulation

Technologically, earliest single-root simulation modeling activities date from the 1970s when computers became generally accessible to plant and soil scientists, but, conceptually, these nutrient uptake simulations can be traced to Bray[7]. Bray envisioned nutrients as being mobile in the soil solution, moving toward the sorbing surface of a root. In the early 1960s, separate papers by Bouldin[11] and Barber[3] built upon Bray's ideas by proposing two simultaneous processes, mass flow and diffusion, governing nutrient movement to the root surface. Both authors present the concept of mass movement in the convective flow of water created by plant transpiration that carries ions present in the soil solution to the root surface. Net diffusion of ions from the surface of the soil particle to the root, movement by random kinetic (Brownian) motion, was hypothesized to occur when root demand or uptake activity exceeded the supply from mass flow. In these circumstances, the root creates a zone of lower concentration in its rhizosphere, which becomes the driving force for diffusion; the lower the nutrient soil solution concentration relative to the plant requirement, the more important the diffusive flux mechanism becomes. Jungk[42] notes that Barber's presentation of mass flow and diffusion has led many to regard these as separate processes, but these processes are interdependent and one cannot technically calculate separate contributions for each mechanism[57].

All published single-root mechanistic simulation

Table 2. Application of mechanistic nutrient uptake models to the study of plant-soil K dynamics.

Plant Species	Subject	Model	Reference
Allium cepa L.	Effect of two soil moisture levels on K uptake (pot study)	Cushman-Barber	Kuchenbuch et al., 1986
Glycine max L.	Effect of mechanical impedance on root morphology and nutrient accumulation (sand culture)	Claassen-Barber	Peterson and Barber, 1981
	Cultivar variation in K accumulation from two soils	Cushman-Barber	Silberbush and Barber, 1983a
	Sensitivity of model parameters to change (computer study)	Cushman-Barber	Silberbush and Barber, 1983b
	Effects of soil bulk density and P addition on K uptake (pot study)	Cushman-Barber	Silberbush et al., 1983
	Five cultivars grown in soil with vertically stratified K availability (field study)	Cushman-Barber	Silberbush and Barber, 1984
Gossypium hirsutum L.	Bio-available fixed K; K/N interactions and influx (pot study)	Cushman-Barber	Brouder and Cassman, 1994a
Oryza sativa	Effect of soil type and cultivar on K uptake (pot study)	Cushman-Barber	Teo et al., 1992
Pinus elliottii Engelm.	Uptake from low K supplying soils and comparison of models (pot and field studies)	B-N-T / Cushman-Barber	Van Rees et al., 1990a
	Competition with grass for K and P at high and low initial concentrations (pot study)	COMP8	Smethurst and Comerford. 1993b
	Effect of irregular root system array on K accumulation (field study)	COMP8	Comerford et al., 1994
Triticum aestivum L.	Effect of Ca and NO_3^- on K accumulation (solution culture and soil pot study)	Bouldin	Bouldin et al., 1992
Zea mays L.	Temperature effects on soil and plant parameters (pot study)	Claassen-Barber	Ching and Barber, 1979
	Genotypic differences in root characteristics and vertically stratified K availability (field study)	Claassen-Barber	Schenk and Barber 1980
	Relationship of CEC to K availability (pot study)	Cushman-Barber	Shaw et al., 1983
	Moisture effects on root growth and K and P accumulation (computer study)	Cushman-Barber	Barber and Mackay, 1985
	Efficiency of placement of fertilizer K and P (computer study)	Cushman-Barber	Kovar and Barber, 1989
	K availability at 5 pH levels	Cushman-Barber	Chen and Barber, 1990
Multi-species or unspecified	Characterization of rhizosphere K delpletion with distance from the root surface (pot study)	Cushman-Barber	Claassen et al., 1986
	Effect of four soil pH levels on K and P uptake	Cushman-Barber	Li and Barber, 1991
	Bio-available fixed K; separate D_e values for exchangeable and fixed K pools (pot study)	Cushman-Barber	Meyer and Jungk, 1993

models that have been applied to K uptake contain an algorithm for diffusion and mass flow (**Table 1** and references contained therein). Typically, the contribution of mass flow to the net movement of nutrients to the root surface is estimated as:

$$F_m = v\ C_l \quad [1]$$

where v is the mean rate of plant water use (transpiration) and C_l is the ion concentration in the soil solution. Supply by diffusive flux is characterized as:

$$F_d = D_e\ \delta C_s/\delta r \quad [2]$$

where D_e is the effective diffusion coefficient for the solute, C_s is the concentration of ions in the solid phase that readily replenish C_l, and r is the radial distance from the root. The D_e is a function of ion diffusivity in water, a constant for each nutrient, corrected for the volumetric water content of the soil, the impedance, and the buffer power of the soil. The volumetric water content determines the fraction of the cross sectional area available for diffusion, while the impedance or tortuosity allows for the diffusion distance of the ion as it follows a tortuous path through water within soil pores. Buffer power reflects the ability of non-solution-phase nutrient ions to replenish soil solution levels as the root depletes this pool.

For conservative systems where nutrient loss mechanisms such as leaching, volatilization, and microbial immobilization are negligible, quantification of mass flow and diffusive flux coupled with a function to describe root system absorptive power can be used to predict nutrient uptake. The most widely used models for describing root absorptive power are kinetic with uptake rates linked to the external ion concentration[55]. It has long been observed that the relationship between the rate of

influx at the root surface and the external ion concentration is not linear[33]. Epstein and Hagen[34] applied terms from enzymology and Michaelis-Menten kinetics to describe the relationship, and with the exception of the Bouldin model, all models listed in **Table 1** rely on this concentration-dependent characterization of ion transport at the root surface. For external K concentrations in the range of 0.0 to 0.2 mM K, uptake has been observed to increase curvilinearily as a function of the external ion concentration in solution (C_l) and asymptotically approach a maximum accumulation rate (**Figure 1**), suggesting that influx can be modeled with the Michaelis-Menten equation:

$$In = I_{max}(C_l - C_{min}) / [Km + (C_l - C_{min})] \quad [3]$$

where I_{max}, Km and C_{min} represent the maximum rate of solute influx, the Michaelis-Menten constant, and the soil solution concentration where net nutrient influx ceases, respectively[4]. The single root models assume that flux to the root surface equals the rate of net solute uptake

$$Fm + Fd = In \quad [4]$$

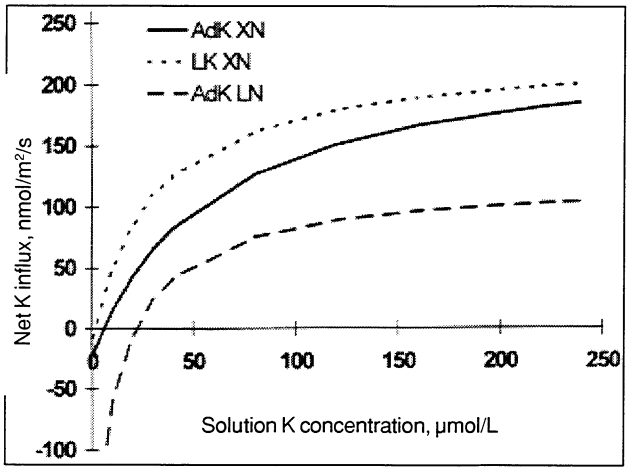

Figure. 1. Relationship between external solution K concentration and K influx of cotton roots grown in a nutrient environment of adequate K with excessive N (AdK XN), adequate K with limited N (AdK LN), and limited K with excessive N (LK XN). Respective estimated values for Michaelis-Menten parameters I_{max}, Km and C_{min} are: 233 µmol/m²/s, 63 µmol/L, and 5.6 µmol/L (AdK XN); 225 µmol/m²/s, 31 µmol/L, and 1.1 µmol/L (LK XN); and 120 µmol/m²/s, 35 µmol/L, and 21.6 µmol/L (AdK LN). Data from a solution depletion experiment described in Brouder and Cassman[17].

Early models such as the Baldwin-Nye-Tinker (B-N-T) model (**Table 1**) simulated nutrient uptake for a single root growing without competition for the soil's nutrient supply. Later models extended the single root case to a growing root system where absorptive potential increases with time. The Barber models (**Table 1**) assume that uptake by each new root starts at a progressively later time during the growth period. To characterize the changing geometry of a growing root system, model input parameters include an initial root length, a mean root radius, and a growth rate constant reflecting the new root growth during the uptake period. The COMP8 model extends the single root system model to a multi-species model with root systems competing for a single nutrient ion, while the Bouldin and Silberbush et al. models simulate uptake of multiple ions by a single plant species (**Table 1**). For specific details on mathematical solutions to equation [4] and discussion of related boundary conditions and assumptions, see Nye and Marriot[56], Barber and Cushman[5], Barber[4], Bouldin[12], and Yanai[87]. For excellent summary reviews of model algorithms, see Jungk[42] and Silberbush[70].

Contributions to the Study of Plant-Soil Potassium Dynamics

Verification of Early Models and Successful Applications.

Regardless of the theoretical accuracy of a model's components, it is generally considered of limited use until it has been verified with independent data collected from a real system[70]. The initial validation confirms that the processes perceived to be the backbone of the model are, in fact, seminal, the assumptions reasonable, and the unknowns not prohibitive. Initial verification usually involves the statistical comparison of model predictions of nutrient accumulation to independently derived, real system observations of accumulation.

Claassen and Barber[25] document one of the earliest attempts to validate a mechanistic simulation model for K accumulation. To test the Claassen-Barber model, they grew corn seedlings in eight different soil/K-rate combinations under controlled environmental conditions. The regression of predicted and observed K uptake values for 14-day old corn gave a fairly close linear relationship across all soil treatments (r^2=0.87), but the model over-predicted K uptake by 50 percent. Schenk and Barber[68] repeated these calculations for field-grown corn and suggested that the over-prediction resulted from the omission of inter-root competition. Since most plant root systems are presented with a finite volume of soil to mine for nutrients, adjacent roots may eventually have overlapping depletion zones. Cushman and Barber[5, 29, 30, 31, 32] modified the Claassen-Barber model algorithm to account for root density, spatial and temporal variability in nutrient supply and age dependence of root parameters. The resulting Cushman-Barber model was validated with reasonable precision and accuracy for K uptake in pot and field studies with corn[68, 69] and soybean[71, 73, 74]. Claassen et al.[26] carried the model validation one step further by analyzing model component behavior, as well as comparing predicted to observed K accumulation. The authors used thin slicing techniques to obtain soil samples from zones of defined distance from the root surface. Model predictions for rhizosphere K

levels as a function of distance from the root surface closely matched K levels extracted from the thin sections.

Such validation results have been widely interpreted as evidence that the Cushman-Barber model accurately simulates essential component processes, as well as total uptake, and that the model's theories and their mathematical descriptions are a reasonable reflection of plant-soil K dynamics. Rengel[66] cautions, however, that some of these validation studies were biased or confounded by factors such as plant age. Schenk and Barber[68], Silberbush et al.[74], and Claassen et al.[26] combined data from plants of different age. Since the simulation model calculated influx over time for a root surface area that increased with time, strong correlation between predicted and observed K accumulations may have been largely the result of substantial differences in root development as a function of plant age. Rengel[66] recommends calculating the weighted mean squares of deviates for the statistical comparison of observed and predicted accumulation values to remove such confounding effects (after Nissen[54]).

Irrespective of such recent technical criticisms, the early reports of success with the Cushman-Barber model encouraged investigators to move beyond simple validation and apply the model to explore rhizosphere relationships that are either difficult to quantify directly or too time consuming and costly to exhaustively address through plant growth experiments in the greenhouse or field. In the 1980s, intensive modeling activity, much of it conducted by Barber and his associates at Purdue University, generated a wealth of information on numerous topics pertaining to plant-soil K dynamics, particularly for corn and soybean. For example, Shaw et al.[69] examined the role of cation exchange capacity in determining the bio-availability of K. When initial exchangeable K levels were controlled, the authors found that a high cation exchange capacity soil did not supply less bio-available K as had been previously hypothesized. Silberbush et al.[71] modeled K uptake at varying soil bulk densities and identified reduced root growth, and not changes in effective diffusion rate, as the causal agent for reduced plant accumulation at higher soil strengths. After verifying the Cushman-Barber model for K accumulation by onion, Kuchenbuch et al.[45] analyzed the calculations to characterize moisture effects on K movement to the root. Their findings regarding soil water content and the width and steepness of the rhizosphere K concentration gradient provided a theoretical explanation for the common observation that visual K deficiency symptoms often precede the appearance of more generalized drought symptoms as a crop becomes water stressed. Kovar and Barber[43] applied the Cushman-Barber model to calculate the most efficient placement of fertilizer K and determined that placement in the profile and the volume of the soil treated were relatively unimportant for K management relative to P management. Current regional fertilizer recommendations[84] reflect these results, demonstrating the practical applications that modeling activities can have in the derivation of sound agronomic practices.

Beyond evaluation of the relative importance of plant and soil factors in the nutrient acquisition process, mechanistic model manipulation during hypothesis-driven applications can generate numerous new testable hypotheses. Indeed, input data from a successful validation can be altered in computer-based exercises for the single purpose of generating testable hypotheses. Typically identified as "sensitivity analyses", these studies systematically change initial values of individual or groups of input parameters to observe the impact on predicted accumulation (**Figure 2**). Inherently, the interpretation of output from such regimented effort requires caution as parameter interdependence cannot be disregarded and not all parameters are equally amenable to change. Nonetheless, sensitivity analyses hold the theoretical potential of both improving the understanding of the processes being simulated and identifying soil or plant characteristics that have promise for future research efforts to enhance crop K uptake and productivity.

The literature contains numerous reports on mechanistic model parameter sensitivity for K uptake. Silberbush and Barber[72] used the approach to demonstrate the relative importance of Cushman-Barber model parameters that characterize soybean root exploration of the soil volume (e.g. the root growth rate and the mean root diameter) and to hypothesize that root geometry instead of uptake kinetics should be the focus of attempts to identify roots with enhanced K uptake efficiency. Other sensitivity analyses for the Cushman-Barber model and the COMP8 model applied to widely varied plant-soil systems have tended to support this suggestion. Investigations of K accumulation by slash pine seedlings, grown alone and in competition with other species[82], and flooded rice[80] also identified root length and diameter as highly sensitive and potentially dominating parameters controlling K accumulation. Similar results were reported for cotton K accumulation across a range of initial K soil supply and plant K absorptive potential (**Figure 2**; Brouder and Cassman[17]). Direct evaluations of the relationship between genetic differences in these root attributes and genotypic K accumulation efficiency for cultivars of soybean[72], corn[68] and cotton[16, 18] have largely substantiated the sensitivity-analysis generated predictions.

Progress through Failure: New Challenges and New Models

In the effort to mechanistically describe plant-soil K dynamics, advances in knowledge have been born from failure as often as from success. Indeed, a wholly verified model would be of limited interest beyond use as an educational tool in that further applications would generate fully anticipated

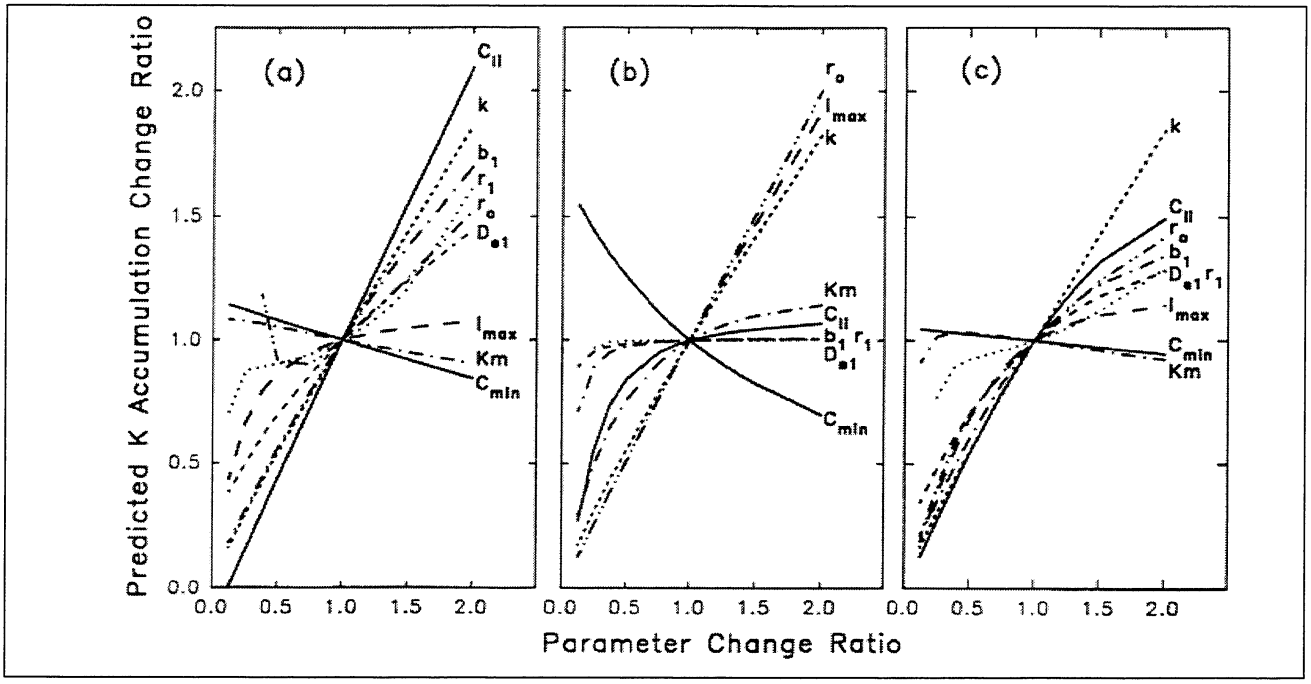

Figure. 2. Analysis of sensitivity to change of selected Cushman-Barber model input parameters for K accumulation by cotton from a vermiculitic soil. Results are expressed relative to K uptake under initial conditions of (a) restrictive soil K supply and strong root absorptive potential, (b) plentiful soil K supply and root absorptive power restricted by nitrogen deficiency, and (c) plentiful soil K supply and strong root absorptive potential. The parameters are the initial K^+ concentration in the soil solution (C_{li}), the soil buffer power (b), the effective diffusion coefficient of K^+ in the soil solution (D_e), the root growth rate (k), the mean half distance between roots (r_1), the mean root radius (r_o), the maximum rate of K influx (I_{max}), the Michaelis-Menten constant (Km), and the soil solution concentration at which net influx ceases (C_{min}). See Brouder and Cassman[17] for initial input values of all model parameters. Absolute predicted K uptake under initial conditions was (a) 0.46, (b) 1.03, and (c) 1.84 mmol K/pot.

results, confirming original theories, but not necessarily suggesting new ones. However, we are far from having a complete mechanistic description of nutrient uptake. For K accumulation, early models such as the Cushman-Barber model, which has been widely successful in terms of sheer number of applications and literature citations, contain numerous, easily assailable assumptions and unrealistic boundary conditions. In addition to the assumption that root influx equals soil solution flux (equation [4]), early models assume that other solutes do not interfere with target ion movement in the soil or across the root plasmalemma. In the validation and application studies thus far reviewed, real constraints on soil K supply were minimal while concentrations of other cations and anions were optimized. In more recent investigations, the focus has shifted to low fertility or physiologically unfavorable situations that may violate one or more critical assumptions. The intent of these investigations is to move simulations outside of the strict boundary conditions within which the model performs well for the purposes of assessing robustness and identifying components or attributes that require refinement or complete mechanistic redefinition.

The first application of the Cushman-Barber model to extremely low K supplying siliceous soils found substantial under-prediction of K accumulation by slash pine seedlings[82]. The authors cited the assumption that roots and root hairs are smooth cylinders with undifferentiated uptake kinetics and the exclusion of the mycorrhizal surface area from the characterization of root exploratory geometry as critical model shortcomings. In a companion note, Van Rees et al.[83] also identified potential difficulties with the mathematical definition of soil buffer power. For K, the Cushman-Barber model assumes that soil buffer power is estimated by the change in the concentration of ions adsorbed onto the soil exchange complex, C_s, per unit change in the concentration of ions in the soil solution C_l [dC_s/dC_l]. Van Rees noted that this definition could only be valid when adsorbed ion concentrations are significantly higher than the C_l. For K in low fertility situations or in sandy soils with low fixation potential, this assumption may often be violated. The algorithm used in the earlier B-N-T model[56,57] characterizes buffer as the change in total diffusable ion (solution plus sorbed) per unit change in the soil solution. This definition may be more generally applicable. As in the Cushman-Barber model, however, the B-N-T model assumes a constant b derived from a linear relationship between solid and solution K phases. Van Rees[83] argued that for low fertility, siliceous soil, the K adsorption isotherm is likely to be non-linear within the concentration range experienced by roots during periods

of active uptake. The COMP8 model corrects for this inaccuracy by introducing the ability to vary buffer power during simulation which has been demonstrated to improve model predictions for K accumulation from siliceous soils[77].

Similar difficulties have been encountered in the attempt to use the Cushman-Barber approach to the estimation of buffer power for soils with fixed K reserves and the potential to fix added K. Non-exchangeable or "fixed" K can be released to the labile or plant-available pool when exchangeable and soil solution K are reduced by plant uptake[36, 78]. Studies on K cycling and cropping system K budgets have demonstrated that the contribution of fixed K reserves to plant K status can be substantial[39, 44]. Nonetheless, for the purpose of modeling, Barber[4] has maintained that solution K^+ concentration beyond the root hair zone remains sufficiently high to prevent significant release of K from adsorption interlayer sites of K-fixing minerals in most soils.

Two recent studies have addressed this assumption. Meyer and Jungk[52] used a continuous percolation extraction to derive separate effective diffusion coefficients and soil buffer powers for K fractions rapidly (exchangeable) and more slowly (non-exchangeable) released by a loess-derived luvisol. Model predictions closely matched observed wheat and sugar beet K accumulations, when plants were assumed to accumulate K simultaneously from both fractions. When fixed K contributions were included, model simulations also improved for vermiculitic soils with high fixation potential. Brouder and Cassman[17] found the Cushman-Barber model substantially under-predicted cotton K accumulation when soil buffer was estimated from the relationship between solution and exchangeable K levels (r^2=0.87; regression coefficient=0.57 for the regression of predicted on observed observations). Predictions improved significantly (r^2=0.87; regression coefficient=1.16), however, when b was estimated from a Langmuir-type, non-linear adsorption isotherm. A more detailed examination of the model predictions from this study reveals that solution K concentrations in the rhizosphere would be much less depleted throughout the uptake period and inter-root competition reduced if fixed K reserves were contributing to replenishment (**Figure 3**). While neither study definitively identified fixed K contributions by measuring K concentrations in the rhizosphere, accounting for plant-available fixed K clearly warrants further consideration for mechanistic modeling of K acquisition. Furthermore, as with the low fertility siliceous soils, adsorption isotherms for vermiculitic soils may be distinctly non-linear within the range of solution concentrations that were observed during an accumulation interval. Thus, a variable soil b power algorithm, such as that offered by the COMP8 model, should also improve K uptake simulations.

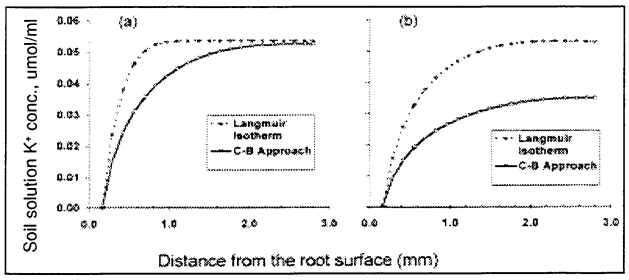

Figure. 3. Cushman-Barber model calculated K concentrations in the vicinity of cotton roots at 12 (a) and 24 (b) days in a 24-day nutrient uptake simulation. At each time, model predictions for rhizosphere K concentrations were generated using either the standard Cushman-Barber approach of estimating soil buffer power from the relationship between solution and exchangeable K levels (C-B Approach) or estimating soil buffer power from a Langmuir non-linear adsorption isotherm (Langmuir).

Potassium behavior in soil and K bioavailability are also strongly influenced by the chemical composition of the soil. Potassium in soil interacts nonlinearly in exchange reactions with other ions, such as Mg^{2+} and Ca^{2+}. Thus, regardless of documented success, the general approach of the Barber models has been repeatedly and justifiably criticized for simulating uptake of one ion at a time[12, 66, 70]. The Bouldin and Silberbush et al. models focus on this issue. The Bouldin model describes simultaneous uptake of K and Ca, Mg, NO_3, Cl, and SO_4. An additional innovative aspect of this model is that it includes the maintenance of electrical neutrality as an objective for model behavior. However, the Bouldin model addresses actual ion interaction in the soil only. The model separately calculates the root uptake of each cation and anion with a conventional single ion algorithm for each time period. The Silberbush et al. model attempts to overcome this deficiency by accounting for interactions between K and Na, Ca, and Mg during uptake. It emphasizes adsorption due to cation exchange mechanisms, dissolution, and precipitation of $CaCO_3$ and pH changes near the root surface that are controlled by cation/anion influx balance.

The Bouldin and Silberbush et al. models, which diverge significantly from the B-N-T and Barber models, are in the relatively early stages of development and application. Bouldin et al.[13] used the Bouldin model to predict wheat K, Mg, and Ca accumulation and plant tissue ratios. Predicted and observed nutrient content ratios in plant shoots for a range of Ca and NO_3 environments were well correlated (r^2 ranging from 0.65 to 0.92), but regression intercepts were not equal to zero and slopes were not equal to one. These discrepancies likely reflect limitations of using a non-mechanistic characterization of ion uptake at the root surface. The Silberbush et al. model, which is intended to examine salt effects on plant K accumulation, has yet to be verified with independent data.

Summary

Mechanistic models have proved invaluable in advancing our understanding of soil-plant dynamics that govern bio-available K. The majority of mechanistic simulation models contain algorithms for ion movement in the soil by mass flow and diffusion, and a kinetic description of nutrient influx into root tissue. Most successful applications have revolved around the identification of the plant morphological and/or soil factors that dominate the K accumulation process.

Early models were developed and validation studies conducted under optimal fertility/physiological conditions. Recent applications of the early models have focused on the overly simplistic assumptions and boundary conditions, laying the foundation for a new generation of simulation models. The latest models have tended to be significantly more complex, the inevitable outcome of rigorous testing, mechanistic replacement of simple empirical relationships and model evolution. Where the original Cushman-Barber model required only 13 input parameters, the Bouldin model requires over 20 input parameters. The Silberbush et al. model requires many more independently-derived plant and soil factors to account for multi-ion interactions. Doubtless, the application of current models to study K accumulation in situations where soil supply to the root surface exceeds root demand will require further layers of complexity. To simulate K acquisition under these circumstances, investigators must improve the relatively poor mechanistic descriptions of ion transport across membranes and characterize the thus far ignored processes of radial symplastic movement and nutrient translocation within the plant.

Synopsis of Future Research Imperatives

Significant research imperatives relative to mechanistic modeling of plant-soil K dynamics can be identified for both model development and model application.

Model Development

Soil potassium supplying power

Advances in mechanistic modeling will require a better understanding of the interactive processes that control transport of K from various soil pools to the root surface. Both for soils with poor nutrient retention and low K buffering and for soils with large fixed K reserves or strong fixation capacity, we need to reassess model algorithms for solution-phase K^+ replenishment. The inclusion of variable soil buffer power as in the COMP8 model represents a significant advance, but for soils in which non-exchangeable K is important in the annual plant-soil K cycle, a mechanistic description of K^+ release from this pool is required to predict K bioavailability[53]. Reitemeir[65] envisioned K^+ fixation and release as a continuous process with fluxes between low affinity sorption sites on organic and mineral surfaces, peripheral interlayer sites where K^+ becomes more tightly "fixed", and high-affinity, deep interlayer sites between clay sheets. In early attempts to explain K dynamics in soil, the different binding sites were hypothesized to have distinct release kinetics, with rapid exchange kinetics attributed to external planar exchange sites, intermediate kinetics to interior edge sites and slow exchange to interlayer sites[9]. By including such variable release rates in uptake models, model performance should be enhanced for soils with an abundance of vermiculitic, micaceous, or smectitic clay minerals.

The need to include multi-ion effects as attempted by the Bouldin and Silberbush et al. models extends beyond the need to investigate salt effects on plant K accumulation. In the field, bioavailability of K can be strongly influenced by the presence of NH_4^+. Ammonium and K^+ ions have similar size and hydration energy and can compete for interlayer and wedge zones of 2:1 clay minerals[20, 22, 51, 79]. Recent studies have shown that the adsorption of K^+ onto a soil surface is suppressed in the presence of added NH_4^+, while the adsorption of NH_4^+ onto the same surface is enhanced in the presence of added K[18, 47, 48]. In agricultural systems where N, frequently in an ammoniated form, is the primary input, NH_4^+/K^+ interactions warrant further consideration for mechanistic descriptions of soil K supplying power.

The literature is rich with reports demonstrating the effects on K bio-availability of numerous other soil chemical and physical properties, many potentially worthy of consideration in efforts to improve the characterization of soil K supplying power. For example, recent studies have shown that specific soil organic matter fractions, particularly N-rich humic compounds, can hold K^+ against soil adsorption[59] and may be responsible for enhanced K availability that has been observed in manured versus unmanured vermiculitic soils[19]. Organic molecules are also instrumental in the formation of soil aggregates. Horn and Taubner[40] demonstrated that K release from large aggregates was reduced when compared to K release from small aggregates due to an increased length of the diffusion path and a smaller "reactive soil volume" for soils with larger aggregates. Given the number of agricultural acres in reduced tillage management and the high rates of organic inputs that typify both traditional small-scale agrarian systems and newer farming systems that include vertically-integrated intensive livestock production components, mechanistic simulations may also require specific adaptation for organic matter effects on K bio-availability.

Potassium influx, translocation and feedback controls

Current models are much weaker in their characterization of influx than in their description of soil supply. Under field conditions, particularly in agricultural systems, situations where K supply does not control the accumulation process are not

uncommon. When K fertility in the root zone is high or when K supply is not the limiting factor, influx parameters and processes will dominate accumulation (**Figure 2b**), and simple, uniform Michaelis-Menten kinetics for a non-differentiated root system function are too simplistic to be useful. Across the range of external concentrations experienced by a given section of root surface, uptake is likely to be discontinuous, requiring a multiphasic description[55]. Nutrient influx can vary along an individual root and with root age and hierarchy[27, 35, 88]. For K, there can be multiple effects of non-optimal concentrations of other nutrients, including competition for available carriers and impaired membrane function (summarized by Marschner[50]). Again, N should not be overlooked in the consideration of multiple-ion effects as the mechanisms for NH_4^+ and K^+ uptake may be competitive[81], and plant N stress can curtail accumulation efficiency (**Figure 1**). Such interactive effects can be expected to vary in space and time as soil supply and plant demand change, rendering the standard practice of quantification of kinetics in solution culture inadequate. Thus, accurate determination of uptake kinetics remains one of the more intractable and important challenges for simulation modeling.

Current simulation models are fundamentally limited in scope in that they do not incorporate translocation and shoot feedback control mechanisms. Our present understanding of shoot/root physiological interactions is too primitive. Yet, when we are able to incorporate these processes, model complexity will necessarily increase and utilization will become more cumbersome. Indeed, it is important to recognize that improvement of models or model components should always strive to balance accurate mechanistic descriptions of only the most relevant, controlling processes with the assumptions that make the model reasonable to apply.

Model Application

Detractors of modeling point out that the activity itself is inherently risky as it tends to separate scientists from real world events and may ultimately cause users either to overlook the importance of gaps between predictions and reality or even completely ignore them[62]. Yet, so long as it does not supplant real world observations, modeling per se will continue to be an invaluable tool for applied scientists of plant mineral nutrition. Concern about the limitations of natural resources coupled with the advent of high technology into the farmyard is driving a profound change in the way we approach nutrient management in agriculture. Where the management objective in agriculture used to stress purchased inputs to optimize environmental conditions for plant growth, management now emphasizes biological and economic efficiency[67]. Judicious use of mechanistic simulations of K accumulation will be critical to all research activities that attempt to definitively quantify efficiencies in soil nutrient supplying power and plant accumulation and economic thresholds for nutrient amendments.

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Chapter 18:
Soil Fertility Management of Potassium for Cotton—Implications in Plant Nutrition

Jac J. Varco

Plant and Soil Sciences Dept., Mississippi State University, MS.

Introduction

Fertilization serves the purpose of augmenting or replenishing soil fertility. In the short-term, nutrients such as N and S are applied annually to supply the nutritional needs of the immediate crop. Immobile nutrients such as P and K have a greater residual value, thus fertilization in excess of current needs can result in a soil build-up. The feasibility of this strategy depends on fertilizer prices and soil test levels. As soil test levels increase for nutrients such as P and K, the probability of an economical response to fertilization decreases. At greater soil test levels, fertilization may be recommended and justified at a maintenance rate designed to replace what is removed by natural soil losses and crop harvesting. Predicting or recommending a fertilizer rate relative to a soil test level that results in the most economical returns is not a fool-proof exercise. The process may be better described as a means of averting risk associated with a less than adequate supply of the nutrient in question necessary to optimize economical yield.

Fertilization of cotton with K is a rather complex issue partly due to the variety of soils on which it is grown. In addition to K adsorption to the soil cation exchange complex, K exists as a constituent of some primary minerals and the interlayer of clay minerals such as hydrous mica[6]. Generally, deep, coarse sandy soils in areas of high rainfall contain minimal weatherable K-bearing minerals; thus, K is retained primarily in an exchangeable form. The primary exchanger in soils of high sand content is organic matter. Thus, potentially available K on sandy, low cation exchange capacity (CEC) soils closely matches extractable or exchangeable K. The accuracy in using exchangeable K as a sole indicator of availability for soils containing significant quantities of weatherable K-bearing minerals is reduced since routine soil tests do not account for potential release from these sources. Finer-textured soils also vary in the capacity to which K applied in fertilizer is fixed or held in nonexchangeable form. Predicting K availability from these soils is difficult due to varying degrees of mineral weatherability and factors such as K diffusion rate and adsorption site affinity for K. The K fixation capacity also affects the optimal fertilization rate[27].

Some of the earliest reports of K deficiency on cotton were on Coastal Plain soils of the southeastern U.S.[13]. These soils are primarily sandy in nature and contain relatively low levels of weatherable K-bearing minerals. The expression of deficiency symptoms became known as cotton rust. Primary symptoms were described as a yellowish white mottling between the veins of older leaves. As the deficiency progressed, these spots became necrotic with brown specks and were more numerous along leaf margins and tips. The leaves eventually turned reddish brown and defoliated prematurely. This resulted in bolls not reaching full development and subsequently not opening[9].

Deficiencies of K which occur later in the growing season express symptomology first on the youngest leaves[25, 41]. This typically has been found for modern faster-fruiting varieties when a heavy boll load is present. Thus, it is important to ensure high K availability throughout the fruiting period. Efforts to manage late-season K deficiency have included foliar sprays[32], and various banding methods[43, 44] of K fertilizer as well as a re-evaluation of soil test levels and recommended fertilization rates.

Nutritional Potassium Requirements

Many researchers have attempted to identify critical or sufficiency levels of K in cotton plant tissue. This has been difficult due to the dependency of tissue K levels on plant age and leaf position. According to early literature on this topic[20], petioles should have 41.0 g K/kg and leaves from 8.0 to 20.2 g K/kg at early boll set (90 days). For this same stage of growth, the University of Arkansas uses values of 45.0 g K/kg for petioles and 13.0 g K/kg for leaves to determine adequacy[3]. Early research by Maples and Keogh[24] found a critical K concentration range of 14.8 to 20.2 g K/kg for the topmost fully expanded leaves, depending on variety and location. In Mississippi, Hsu[17] suggested a critical concentration of 10.0 g K/kg for recently matured leaves sampled near early bloom, however, Nichols[30] found 12.8 g K/kg was necessary. Bednarz and Oosterhuis[4] recently reported 80 g K/kg for non-deficient petioles and 40 to 60 g K/kg for deficient petioles; and 30 g K/kg for healthy leaves and 20 g K/kg for deficient leaves at the first sign of deficiency 4 days after first square. This research was conducted in a growth chamber using a sand growing media. Cassman et al[8] working on a Grangeville sandy loam (coarse-loamy, mixed, thermic fluaquentic Haploxeroll) in California indicated that a petiole K concentration of 30 g K/kg at peak bloom was necessary to maximize lint yield. Although relationships between leaf or petiole K and yield are evident in single-factor experiments[1, 8, 17], their utility is limited to some degree by variability.

Petiole and leaf K concentrations are greatest near early square and decrease with plant development[18] and differ relative to canopy position[4]. Apical immature leaves and old mature leaves show the greatest decline in tissue K concentration from early flowering to half grown bolls[18]. Verticillium wilt (*Verticillium dahliae*) can complicate interpretation since tissue K levels can be reduced by the infection[11]. The number of nodes with deficient petioles and leaves increases from top to bottom as the deficiency becomes more severe[4]. The developing bolls have been shown to be the greatest K sink[4, 8], resulting in a decline in K concentration in the apical immature leaves and an expression of K deficiency symptoms. A decline in root function including K uptake during fruiting is likely, as the developing bolls are a photosynthate sink also.

Bennett et al.[5] noted that the K concentration of old mature leaves and petioles was most responsive to K fertilization. Hsu[18] reported a strong relationship between soil test K and the K concentration of both young mature and old leaves. Tissue K concentration of young, mature and old leaves sampled at early flowering has been shown to be correlated with soil test K levels[17]. Additionally, the level of soil test K required for a given leaf K concentration was found to increase with an increase in CEC. Correlations between leaf K and exchangeable K were improved if silty clay and clay loam soils were categorized separately. Some states, including Mississippi, utilize soil CEC to establish sufficient soil test K levels[12].

With the adoption of short-season varieties there has been some concern for adequately meeting nutritional uptake requirements. As a result of breeding, there has been a shift in dry matter production with modern varieties partitioning a greater percentage into fruit[26]. Bennett et al[5] found K uptake ranged from 7 to 19 kg per 100 kg lint produced; K uptake proportionally increased relative to yield with increasing fertilizer K rates. Recently, Mullins and Burmester[28] reported an average K uptake requirement of 15.3 kg K per 100 kg lint with no difference in uptake among four cotton cultivars. Potassium uptake relative to yield does not appear to have been altered greatly through breeding, but uptake rates may be greater due to the shorter growth period. Keino et al[21] showed faster depletion of solution K by a late-maturing variety as compared to an early maturing variety, but the late maturing variety had a greater affinity for K at low solution levels.

Potassium Effects on Magnesium Nutrition

Soils containing marginal available Mg may be less responsive or respond negatively to K fertilization. Davis-Carter et al.[10] observed induced Mg deficiency symptoms from K fertilization. Petiole Mg concentrations declined to less than 4.0 g Mg/kg at flowering with K fertilization on a Coastal Plain soil in Georgia. Generally, Coastal Plain soils have low Mg levels compared to other cotton growing regions due to high sand content, low CEC, and high water permeability. Mississippi River Delta soils generally contain high Mg levels. Adeli[1] showed declining leaf and petiole Mg concentrations with increasing fertilizer K rates, but no Mg deficiency symptoms were observed with leaf Mg concentrations of 4 g Mg/kg or greater. Thus, on soils having marginal Mg supplying capacity, K and Mg fertilization may be more appropriate than K fertilization alone.

Potassium Fertilization

Potassium fertilization research has been conducted for many years in most of the cotton growing states in the U.S. In Arkansas, Maples and Beacher[22] dicussed early research in cotton fertilization. Generally, fertilizer K responses were found, but the range in soil test levels at which they occurred was wide[24]. A significant observation by Maples and Keough[24] was the expression of K deficiency symptoms early in the growing season and to a greater degree for an early maturing variety than a full season variety. The incidence of late-season K deficiency has coincided with the widespread adoption of early season varieties.

Early research in Mississippi by Grissom[14] and Grissom and Spurgeon[15] indicated variable cotton response to K fertilization. This research was conducted on private farms and university branch experiment stations. These results were likely caused by variable soil K supplying capacity as well as prior fertilization practices. Pettiet[35] similarly found variable response to K fertilization on Mississippi River Delta soils. Cotton fertilized with K was taller and more vegetative, and deficiency symptoms, when noted, were only in localized areas within non-fertilized plots. Leaf symptoms were fully expressed late in the season, and many shed prematurely. Additionally, many bolls were small and failed to open. Pettiet[35] concluded that separating sandy loam and silt loams soils for soil test calibration would improve correlations and, thus, recommendations. Thom[42] working on Mississippi River Delta soils testing high in K found a consistent response to K fertilization across soil types. These results imply that soil test K calibrations may need to be reevaluated.

In Alabama, Rouse[38] demonstrated the importance of soil K buildup for sustaining cotton productivity. Bennett et al.[5] noted increased seedcotton yield and boll size in response to K fertilization. Also, fertilizer K rates of 280 kg/ha and greater delayed maturity due to enhanced vegetative growth. The check plots experienced earlier cutout and showed severe signs of K deficiency to the point of premature defoliation.

Potassium deficiency has long been a problem in California. Stromberg[41] identified soils derived from granitic alluvium as the most problematic. These soils contain an abundance of mica type minerals as well as montmorillonite and vermiculite

type clays. The term late-season was first used by Stromberg[41] to describe K deficiency symptoms expressed on younger leaves. The presence of verticillium wilt in some of these soils has complicated the issue[16].

Increasing incidence of late-season K deficiency has resulted in greater research emphasis on cotton K requirements and response to soil test K levels and fertilization. Cassman et al.[7] working on a Grangeville sandy loam soil found distinct differences between what was considered a K-efficient cultivar and one which was not. The K-efficient cultivar had greater yield across broadcast incorporated K rates of 0 to 480 kg/ha. Maximum seedcotton yield was predicted at a leaf tissue K concentration at early bloom of 14.5 g K/kg for the K-efficient cultivar compared to 17.8 g K/kg for the non-efficient cultivar. Under non-K limiting conditions, however, yield potential for both cultivars was similar. Response to fertilizer rates as great as 480 kg K/ha is typical on soils derived from granitic alluvium having a high K-fixation potential[34]. Additionally, a trend in declining yields from year to year was observed on these soils if fertilizer K was not applied[8].

Silvertooth et al.[39] evaluated cotton yield response to fertilizer K on a variety of soils in Arizona. All soils evaluated contained K-bearing mica and except for one, had exchangeable soil K of at least 150 mg K/kg. No differences in yield, lint quality, and K nutrition were found on the young fertile soils included in this study.

Establishing cotton yield response to soil test K levels ranging from deficient to sufficient or greater is necessary for calibration. In Mississippi, Hsu[17] found maximum cotton yield at soil test K levels of 96 mg K/kg for sandy loams, 107 mg K/kg for silt loams, and 140 mg K/kg for clay soils. These values were based on the Mississippi soil test extraction procedure as described by Pettiet[35]. An idealized lint yield response curve to soil test K level is shown in **Figure 1**. Maximum lint yield on this soil would be predicted at soil test K level near 165 mg/kg. This type of relationship is used to determine the probability of obtaining a response to fertilization or an increase in soil test K. The University of California uses a similar response curve and has established a critical soil test K (ammonium acetate or Mehlich 3) level of 110 mg K/kg [27]. This is considered a threshold value at which a response to added fertilizer K would be less than 10 percent.

Application Methods

Early research on broadcast versus band placement of fertilizer K generally showed little difference between the two methods[2, 23, 45]. In these studies, treatments were either N, P, and K or P and K combined, thus, individual nutrient effects could not be evaluated. Maples and Keogh[23] found band placement of fertilizer increased seedcotton yield (204 kg/ha) only on one out of four soils tested. Re-

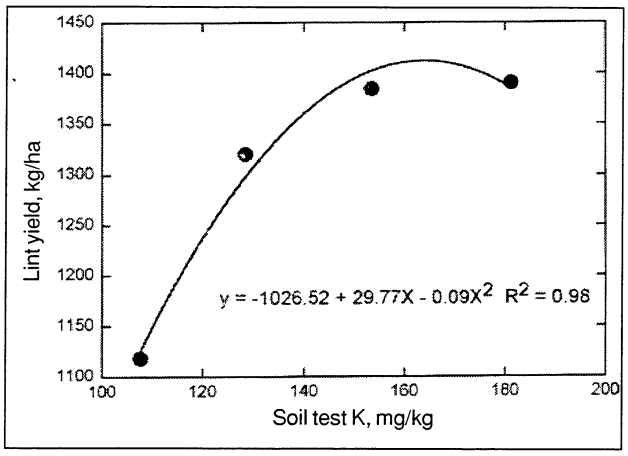

Figure 1. Lint yield response in 1995 to established soil test K levels on a Morganfield silt soil located near Yazoo City, MS (Varco, 1997, unpublished).

cent research by Adeli[1] in Mississippi conducted on a Morganfield silt (coarse-silty, mixed, nonacid, thermic Typic Udifluvent) showed a quadratic response to broadcast incorporated K rates across a 3-year period (**Figure 2**). Banded K response was no different than broadcast rates up to 68 kg K/ha, but yield declined with 102 kg K/ha. Yields increased synergistically with a combined application of 34 kg K/ha banded and 136 kg K/ha broadcast. Smith and McCutchen[40] found additional yield produced when broadcast N, P, and K fertilization was combined with a band of 8.4 kg N/ha, 3.7 kg P/ha, and 7.0 kg K/ha placed 5 cm below the planted seed, but a specific response to K could not be inferred from their results. Seedcotton yield was increased 342 kg/ha from this combined application on a Grenada silt loam (fine-silty, mixed, thermic Glossic Fragiudalf) in western Tennessee.

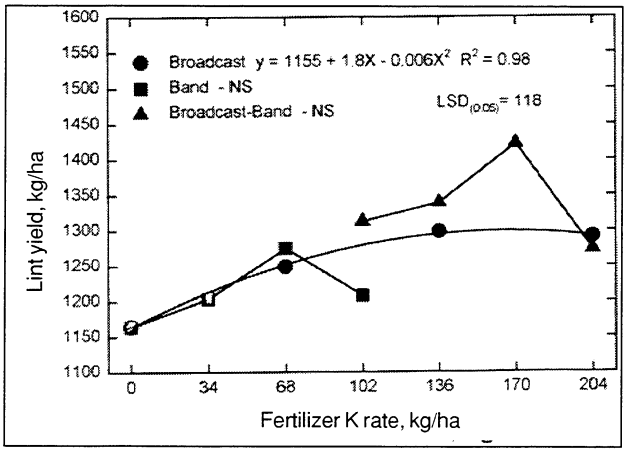

Figure 2. Average lint yield response for the years 1991-1993 to rates of fertilizer K applied as a 10 cm X 10 cm band, broadcast incorporated, and a combination of the two on a Morganfield silt soil located near Yazoo City, MS.

Adeli's[1] work showed the effectiveness of broadcast fertilization on K nutrition. A linear increase in petiole and leaf K at early flowering resulted

from broadcast incorporated rates. Exchangeable soil K increased linearly and was correlated with leaf and petiole K at early flowering. The Morganfield (CECC=11.1 $cmol_c/kg$) soil required approximately 2.2 to 2.3 kg fertilizer K/ha to increase soil test K 1 kg/ha in the 0- to 15-cm depth. Total K uptake also responded linearly to broadcast rates of K.

Tupper et al.[43] evaluated the response of four varieties to broadcast, deep banding, and a 50/50 split in K rate between these two methods of fertilization. The research was conducted on a Beulah (coarse-loamy, mixed, thermic Typic Dystrochrept)-Bosket (fine-loamy, mixed, thermic Typic Hapludalf) very fine sandy loam soil association for four years. The greatest response was to the first incremental rate of 75 kg K/ha and no further response occurred with rates greater than 112 kg K/ha. Neither deep banding nor a combination of deep banding with broadcasting resulted in an average yield response significantly greater than broadcasting alone across the 4-year period.

Soil test correlation-calibration research has historically focused on topsoil, but the intent of in-row deep banding of fertilizer is to build up subsoil fertility, albeit only in a narrow vertical zone. Mullins et al.[29] working in Alabama on a Norfolk fine sandy loam soil with a traffic pan found in-row subsoiling increased growth and yield of cotton compared to a non-subsoiled check. Broadcast K in combination with subsoiling increased lint yield and total K accumulation greater than broadcast K application or in-row subsoiling with deep banding of K. As part of this same study, Reeves and Mullins[36] reported increased leaf K (recently matured leaf blades sampled at early flowering) for all K treatments compared to the control. Leaf K deficiency symptoms were expressed more in subsoiled plots and were most severe when fertilizer was placed deep. In-row subsoiling and broadcast fertilization resulted in yield increases, while deep banding of K did not.

Foliar fertilization with K has been suggested to supplement K needs of fast-fruiting cotton varieties during flowering and boll development[32]. A positive response to 44 kg KNO_3/ha applied foliar in 11 kg/ha increments at 2, 4, 6, and 8 weeks after first flower was found. Subsequently a Beltwide study was initiated to evaluate foliar K fertilization across a broad range of environmental and soil conditions at 10 to 13 sites from 1991 through 1993[31]. Averaged across all sites and years, soil test recommended fertilizer K rates increased seedcotton yield 111 kg/ha, and a 2X recommended rate increased yield 162 kg/ha. Foliar KNO_3 at 11 kg/ha applied four times at 10 to 14 day intervals beginning after first flower increased seedcotton yield 75 kg/ha in combination with recommended fertilizer K rates and 24 kg/ha in combination with the 2X fertilizer rate. This indicates response was not specific to foliar fertilization, although it could be beneficial if soil supply is not adequate.

Jones and Jackson[19] did not obtain a significant yield increase (146 kg/ha seedcotton) to foliar KNO_3 fertilization across a 3-year period on a Marietta fine sandy loam (fine-loamy, siliceous, thermic Fluvaquentic Eutrochrept) initially testing high in K. Increases in leaf and bur K concentrations following foliar K fertilization were not significant. The lack of significance, especially for leaves, was attributed to possible translocation of K to other plant parts during the 7-day interval between spraying and tissue sampling. In a comprehensive review of the literature on foliar K fertilization of cotton, Oosterhuis[33] indicated that the response across the Cotton Belt has been variable and unpredictable, and conditions for the greatest probability of a response would include a low soil K status and a history of late-season deficiency.

A recent economic analysis showed foliar KNO_3 to be profitable on low K testing soils even when combined with the recommended soil applied fertilizer K[37]. The researchers suggested that foliar K could be used as a supplement to soil applied K in areas with known late-season K deficiency. The University of Arkansas has developed a protocol for foliar fertilization based on weekly petiole analysis[3]. Approximately 5 weeks into flowering, producers are warned of impending K stress, and at 6 weeks, foliar fertilization is recommended if the condition persists. Oosterhuis[33] summarized the work on suitability of K sources for foliar feeding and indicated a general positive response to KNO_3, K_2SO_4, and $K_2S_2O_3$, while KCl did not increase yield, and K_2CO_3 decreased yield.

Summary

It is evident that the need for K fertilization for cotton has increased based on recent reported yield responses and incidences of known K deficiencies in producer fields. Tissue testing can improve understanding of plant K needs, but results vary due to leaf position sampled and stage of growth as well as environmental influences. Thus, a range in sufficiency rather than a critical concentration may be more appropriate. Soil testing is essential for determining fertilizer needs. Exchangeable soil K and CEC are useful in establishing soil test categories. Other important considerations are realistic yield potential and K fixation potential for appropriate soil types. Broadcast incorporated K fertilizer is an effective method of improving K nutrition of cotton. Banding in combination with broadcast application may be appropriate for high yielding soils as well as soils with a high K fixation potential. Band placement, however, will have to be economically justified due to increased equipment, fuel, and labor expenditures. Foliar fertilization is useful under certain circumstances. Supplemental foliar K is most likely justified when a developing heavy boll load causes tissue K concentration to decline below a sufficiency level and the crop is too mature for soil applied K to be effective. Additionally, cotton grown on soils testing low

in K may benefit if soil applications are not adequate.

Synopsis of Future Research Imperatives

Soil test correlation and calibration refinement across varied soil types is justified considering widespread adoption of high-yielding, early-maturing varieties and availability of variable rate technology. Consideration in soil testing should be given to grouping soils based on CEC and K fixation capacity where applicable to establish soil test categories. Establishing ranges in tissue K sufficiency at or near early flowering is essential for developing in-season fertilization guidelines. There is a lack of knowledge on fertilization relative to realistic yield goals. Recent advancements in spectral imaging of crops should enhance our capability of mapping areas of K deficiency within fields. Coupling this information with variable rate fertilization should increase precision in fertilization. This will require the development of a spectral index which is correlated with tissue K concentration. Both foliar and soil applied fertilizer could then be guided based on soil test values and adjustments for areas with known K stress as well as yield potential.

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Chapter 19:
Potassium Deficiency and Cotton Fiber Development

William T. Pettigrew

USDA-ARS, Cotton Physiology and Genetics Research Unit, Stoneville, MS 38776

Introduction

Potassium is one of the major nutrients needed for adequate plant growth and development[22]. Although not a constituent of any specific cellular component, structure or organelle, K, nevertheless, is essential for the operation of many plant biochemical processes. While there exists a large knowledge base regarding the function and beneficial aspects of K in plant growth, unfortunately, there are still reports of K deficiency developing in cotton fields across the U.S. cotton production belt[16]. These deficiency symptoms have occurred over a diverse range of environments (consisting of soils possessing varying degrees of native K supplying power) and cotton genotypes. This report will focus on the ways that a deficiency in K can alter fiber development in cotton.

Plant Growth

One cannot separate the effect that K has on fiber development from the effects that it produces upon the overall growth of the plant. As a K deficiency in cotton progresses throughout the course of a growing season, the plants will eventually express the classic chlorotic leaf symptoms associated with many nutrient deficiencies in plants. Often times, the symptoms appear in older lower canopy leaves near the time of cutout, a growth phase characterized by a slowing or cessation of vegetative growth. However, under certain deficiency conditions, the symptoms can show up in young upper canopy leaves and also earlier in the season[2, 16]. Even before these symptoms become apparent, the plant has undergone an irrevocable alteration in its vegetative growth pattern. This alteration is seen as general stunting of the growth for K deficient plants.

Both Cassman et al.[5] and Mullins et al.[15] reported reduced leaf dry weights and total plant dry weights for the K deficient plants, compared to plants receiving adequate K. Although Pettigrew and Meredith[20] did not find significant differences in total plant dry matter between control and K deficient plants, they did report a 12 percent lower leaf area index (LAI) and a 2 percent lower plant height for the K deficient plants. This lack of total plant dry weight differences between the K treatments was accounted for in part by the 14 percent increased specific leaf weight (SLW) found in the low K plants. Although not measured in the respective studies, if a SLW increase for K deficient plants also occurred in the studies of Cassman et al[5] and Mullins et al.[15], then a reduction in leaf area under K deficiency would be even greater than that indicated by the leaf weight differences they reported. These data contrast with the work of Joham[10] who found K deficiency to increase vegetative dry matter production while decreasing the fruiting index in cotton. Most evidence indicates that K deficiency reduces the leaf area per plant[5, 15, 20]. This lower total leaf area coupled with the reported lower photosynthesis per unit leaf area in K deficient cotton[3, 12] indicates that the net effect of a deficient level of K is reduced photosynthesizing capacity per plant.

Lint Yield

The most important aspect of fiber development from the cotton producer's point of view is the final lint yield, because that is the primary determinant of economic return. Because K is an essential plant nutrient, a deficiency can reduce the economic yield of most crops. In cotton, studies conducted in numerous environments across the U.S. cotton production belt have documented the negative impact that a K deficiency can have on seedcotton and lint yields[4, 5, 6, 7, 21]. Mullins et al.[15] also reported a lint yield increase in response to K fertilization, but only when that treatment was coupled with an in-row subsoiling treatment. On the other hand, Minton and Ebelhar[14] detected no significant lint yield differences between 0 and 112 kg/ha K treatments, although they did report reduced verticillium wilt (*Verticillium dahliae* Kleb.) symptoms and a lower Southern root-knot nematode (*Meloidogyne incobnita*) root-gall index in the K fertilized plots. Those studies not reporting a yield response to K fertilization were likely conducted on soils where the native soil K status was sufficient to produce comparable yields.

Cotton yield reductions caused by K deficiency can generally be translated into corresponding reductions in most of the pertinent yield components. Pettigrew et al.[21] reported a 5 percent reduction in individual boll mass from K deficient plants, while Bennett et al.[4] found that more bolls were required to produce a pound of seedcotton from K deficient plants than from plants receiving adequate K fertilization. The number of bolls produced per unit ground area was not addressed in either of those studies. Minton and Ebelhar[14] did not detect individual boll mass differences between K fertility treatments. Lint percentage reductions caused by K deficiencies were reported by Bennett et al.[4], Cassman et al.[6], and Pettigrew et al[21]. Minton and Ebelhar[14] did not find lint percentage to differ sig-

nificantly between K treatments. Individual seed mass was reduced approximately 3 percent in seed from K deficient plants, compared to seed from control plants[14, 21].

Fiber Quality

Fiber quality traits, particularly micronaire (MIC) and fiber length, have always been a concern for cotton producers and processors in the textile industry. Unfortunately, it has not always been easy to simultaneously achieve breeding goals of both yield increases and fiber quality improvement. This situation was particularly problematic for fiber strength which historically demonstrated a negative correlation between fiber strength and fiber yield. More recent breeding efforts seem to have broken any deleterious linkages between fiber quality and yield that may have existed[13]. Now with new high speed spinning machines (rotor spinning) coming on-line in many textile mills, the need for superior quality fiber becomes even more essential[8].

The most common and perhaps most misunderstood fiber quality trait is MIC. Micronaire is used as an estimate of fiber fineness but is determined by measuring the air-flow resistance of a given mass of cotton under prescribed conditions using an air-flow instrument. There is a preferred range of fiber MIC (3.5 to 4.9) for the lint sold by producers. Cotton delivered to market with a MIC falling outside that range is penalized in its selling price with discounts as high as $0.135/lb ($0.297/ kg) for cotton with MIC £2.4. Cotton with appropriate color and trash grades and a MIC in the 3.7 to 4.2 range can be sold for a slight premium price. Since the 1991 introduction of high volume instrumentation (HVI) classing for all cotton lint processed by the USDA Agricultural Marketing Service, additional fiber quality traits, such as fiber strength, have received more attention from the cotton industry. There are now slight discounts and premiums for cotton brought to market with bundle strength falling below or above the base range of 23.5 to 25.4 g/tex. The largest discount for strength is $0.017/lb ($0.037/kg) for cotton with strength of the range 18.4 to 19.4 g/tex, while the highest strength premium is $0.0055/lb ($0.012/kg) for strength ³30.5 g/tex. These discounts and premiums for strength are minor compared to those of MIC.

Similar to the reductions in overall plant growth and lint yield, poor fiber quality development often results from growing cotton under deficient K conditions. The effect on individual fiber quality traits has been inconsistent among the various studies. Whenever there was a significant response to K fertilization, the K deficiency always produced a reduction in the fiber quality parameter. Pettigrew et al.[21] reported that K deficiency caused a 10 percent reduction in MIC and a 5 percent reduction in fiber maturity, a component of MIC. Both of these fiber traits are associated with fiber secondary-wall thickening. Similar reductions in MIC due to low K levels were reported by Bennett et al[4] and Cassman et al.[6], but Minton and Ebelhar[14] failed to find a K effect on MIC. A smaller fiber perimeter (the second component of MIC) was also found under K deficient conditions compared to the control[21]. Reductions in MIC and fiber maturity have also been attributed to possible reductions in assimilate supply available to the developing fiber[18, 19]. Less photosynthate translocated from the leaves of K deficient cotton plants[1] could contribute to the lower MIC and fiber maturity readings.

Bennett et al.[4], Cassman et al.[6], and Pettigrew et al.[21] all reported that K deficiency reduced fiber length. Previously, Dhindsa et al.[9] had demonstrated in ovule culture that malate and K are the principle determinants of osmotic potential needed to produced the turgor pressure for the elongation of the primary cell wall in cotton fibers. Thus, it is easy to envision how an inadequate K supply could result in higher (less negative) osmotic potentials, lower turgor pressures, less primary cell wall elongation, and ultimately shorter fibers.

The effect of K deficiency on fiber strength and fiber elongation (the increased fiber length at the breaking load during a strength test expressed as a percentage of the original length) is not as consistent as some of the other fiber traits. Cassman et al[6] and Minton and Ebelhar[14] reported that fiber from the K deficient plants was weaker than fiber from plants receiving K, but Bennett et al.[4] and Pettigrew et al[21] did not detect fiber strength differences among K treatments. Fiber elongation was similarly affected. Cassman et al[6] and Pettigrew et al.[21] found decreased fiber elongation under K deficiency, while Bennett et al.[4] and Minton and Ebelhar[14] did not detect significant fiber elongation differences.

This inconsistency in the apparent effect of K on fiber strength leads to speculation that K probably exerts only an indirect effect on fiber strength. Bennett et al.[4] previously demonstrated that low K levels accelerated the maturity of the cotton crop and resulted in early termination of reproductive growth. The environmental conditions during the shortened reproductive growth window under low K conditions, compared to the longer reproductive growth under adequate K conditions, may have more to do with the fiber strengths reported than did the actual K levels.

Conclusions

Many of the detrimental effects that a K deficiency produces on fiber development mimic those produced by an insufficient supply of photosynthetic assimilates. Both the depression in yield and reductions in the individual yield components seen under K deficient conditions, could also be produced by reductions in the photosynthate supply[17]. Low levels of photosynthetic assimilates can also produce the same reductions in MIC and fiber maturity[18, 19] often seen in fiber from K deficient plants The stunted plant growth caused by K deficiency

results in less photosynthesizing leaf area per plant[20]. This phenomenon, coupled with the lower photosynthetic rate per unit leaf area seen in K deficient cotton leaves[3, 12] undoubtedly would result in lower amounts of photosynthate available per plant under K deficient conditions. Besides altering the assimilate supply, there are certainly other ways that K can influence fiber development, such as the osmotic role this element plays in determining fiber length. It appears, however, that the disruption to the photosynthate supply available to the developing fiber that a K deficiency causes by lowering photosynthate production and further inhibiting translocation out of the leaf[1] is the principle way that K affects cotton fiber development.

Summary

Over the years, producer reports of K deficiency in cotton (*Gossypium hirsutum* L.) fields have come from every part of the U.S. cotton production belt. Before the leaf develops the characteristic chlorotic symptoms, the general growth of the plant is stunted and total leaf area is reduced. The reduction in overall photosynthesizing area and capacity substantially reduces the amount and quality of the fiber produced. Lint yield reductions of 10 percent or more are not uncommon and can be traced to corresponding reductions in most yield components. Fiber traits associated with secondary wall thickening (micronaire and fiber maturity) are consistently reduced by a K deficiency. Because K serves as an osmoticum in producing the turgor pressure that drives fiber elongation, a deficiency in K can also produce shorter fiber. Potassium deficiency sometimes causes lower fiber strength, but this effect has been quite inconsistent. Reductions in overall photosynthate production and the translocation of that carbohydrate to the developing fruit can explain many of the effects seen in fiber development for cotton grown under K deficiency.

Synopsis of Future Research Imperatives

The understanding of the physical and biochemical aspects of fiber quality development is still quite rudimentary. The physical traits that produce strong fiber are not completely understood. In this paper, it is speculated that a K deficiency induced lowering of the available photosynthetic assimilate supply for the developing fiber is the primary reason for the fiber quality reductions. This hypothesis needs to be confirmed with further experimentation to assess whether fiber carbohydrate levels or activities of pertinent enzymes are altered by a K deficiency. Biotechnology offers the promise of improving various fiber quality traits or altering fiber pigmentation through genetic engineering[11]. Whether the role that K plays in influencing fiber development in the new transgenic cotton is diminished, amplified, or unchanged remains to be determined.

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Chapter 20:
Agronomic Management of Potassium in the 21st Century: Can Plant Physiology Help?

Kenneth G. Cassman

Dept. of Agronomy, University of Nebraska, Lincoln, NE, 68583-0915.

Introduction

World population is projected to exceed 8 billion people by 2020[50], which represents a 52 percent increase since 1990. During this 30-year period, cereal production must increase by nearly 60 percent on existing arable land to meet demands of both population growth and improved diets supported by higher incomes in developing countries[43]. Although an adequate supply of all essential plant nutrients will be needed to achieve this increase, improved K nutrition will become increasingly important for several reasons.

Crop K requirements are greater than requirements for all nutrients except N. Whereas biological N fixation and rainfall provide significant N inputs to terrestrial ecosystems, there are no renewable sources of K in the biogeo-chemical cycle. Crop K uptake is therefore solely derived from existing soil reserves, recycled K in crop residues, and applied K fertilizer. In irrigated systems, irrigation water may also contribute small amounts of K to the overall K balance. Because the native soil K supply is a fixed quantity, a 60 percent increase in food production will require a larger proportional increase in global K fertilizer use. Application rates to soils already K deficient must increase in proportion to higher yield levels, and, in addition, K application will be required in many areas where soils do not presently require K inputs to achieve current yield levels. Increasing K use efficiency in agricultural production systems is therefore necessary to minimize the projected increase in K fertilizer requirements.

Although recoverable mineral K reserves and potash fertilizer production capacity are ample, they are highly concentrated in a few locations within North America, Europe, and central Asia[45]. South, east, and southeast Asia are the regions with the greatest imbalance between exploitable K reserves and K requirements. This region contains 60 percent of world population, but accounts for only 4 percent of world K fertilizer production and less than 8 percent of global K reserves[1]. Imports of K fertilizer to countries in this region increased from 1.0×10^6 t in 1975 to 5.0×10^6 t in 1992, a five-fold increase in 17 years, and imports must continue to grow rapidly to sustain the needed increases in food output.

At issue, then, is how agronomic research in general, and plant physiology in particular can contribute to global food security by reducing costs and increasing returns from K fertilizer application to food, forage, and fiber crops. To address this question, the following review will emphasize plant traits and physiological responses associated with improved crop productivity on soils where K supply is a major limitation. Physiological responses at the whole plant, root, and cellular levels will be highlighted. Comprehensive understanding of these processes and their genetic control are crucial for the development of crop and soil management practices to increase K use efficiency and for the improvement of KUE in crop varieties by conventional and molecular breeding approaches.

An Agronomic Perspective of Potassium Use Efficiency

For the purpose of this discussion, K use efficiency (KUE) is defined as the ratio of economic yield to the available K supply from native soil reserves and applied fertilizer. This definition has a strong agronomic bias because it ultimately requires documentation of cause and effect relationships between crop KUE under field conditions and processes that influence K uptake or utilization within the plant. Crop KUE is determined by both the efficiency with which plant roots acquire K from soil and the effectiveness with which acquired K is utilized by the plant to produce economic yield.

Although there is a large body of published research on processes and mechanisms that influence K acquisition and utilization by plants, most of these studies were conducted in the greenhouse or growth chamber. Few studies have attempted to document cause and effect between these traits and crop performance in the field. Moreover, there are few instances where knowledge of physiological processes has been used to improve crop management or crop germplasm to increase KUE. Cases where attempts have been made to document cause and effect relationships are highlighted in the following sections and promising avenues for future research are discussed.

Potassium Uptake Efficiency

Root Morphology, Root Development, and Root Distribution

Root system architecture influences K uptake rates from a field soil. These traits include root surface area density, which is determined by root length density, root radius, and root hair length and density, and the mean distance between roots which determines the degree to which individual root segments compete for available K[4,33]. Although

genotypic variation has been reported for each of these traits in several crop species and heritabilities appear to be relatively high[38], direct selection for traits that increase K uptake efficiency has not been utilized in plant breeding programs. In part, lack of breeding efforts on root traits reflects the difficulty in developing mass screening techniques for direct selection.

Another problem has been the lack of convincing evidence that selection for specific root traits actually contributes to increased KUE under field conditions. Such verification is critical because pot and solution culture experiments have shown that root morphological traits which favor K uptake can be offset by other root traits that decrease K uptake potential. An example of such counteracting traits was found in two soybean (*Glycine max* L.) genotypes which either had greater root length and a low K uptake rate per unit root surface, or smaller root length and a high uptake rate per unit surface area[40, 41]. Field verification is also important because most pot and nutrient solution studies evaluate K uptake over short periods during vegetative growth stages. Root traits and associated efficiencies measured under these conditions may not accurately predict field performance because morphological traits and uptake parameters can change during the course of plant development, especially during the reproductive growth phase. Moreover, root growth in artificial growth systems is often very different than in a field soil.

In a few cases, obvious differences have been noted in the performance of commercial crop varieties on K deficient soils, and field investigations were conducted to determine the reasons for these genotypic differences in KUE. In one example, two widely grown cotton (*Gossypium hirsutum* L.) varieties performed differently on K-deficient soils in California[10]. Seed cotton yield and K uptake of Acala variety 'GC510' was 30-35 percent greater than for Acala 'SJ-2' in K-deficient soil while yields converged as soil K levels increased. These trends are clearly evident when yields were regressed against an index of available soil K (**Figure 1**).

The response of SJ-2 met the standard of a K inefficient genotype as described by Gerloff and Gabelman[20]: "To be accepted as inefficient under nutrient stress, a genotype must be normal in appearance and must yield approximately the same as efficient strains under optimum supplies of the element." This distinction avoids designating a genotype as inefficient when growth is limited by a physiological limitation or lack of adaptation that does not specifically involve the nutrient in question.

Subsequent studies identified several root and root system traits associated with the higher KUE of the GC510 genotype. The rate of root growth after the onset of flowering was one of these traits[6]. In the 29-day period following peak flowering, root surface area from 0 to 60 cm depth in the K-efficient GC510 increased 100 percent more than that of SJ-2 (**Table 1**). This 29-day interval corresponds to the period of greatest K uptake requirements in cotton. Although most of the genotypic differences in root surface area resulted from increased root length development, root diameter was also significantly greater in GC510. Both root length development and root diameter are sensitive parameters affecting K uptake from soils with strong K buffering capacity such as those in the San Joaquin Valley of California[7]. The genotype differences in root diameter measured in field-grown plants could be detected at an early growth stage when grown in nutrient solution culture[8].

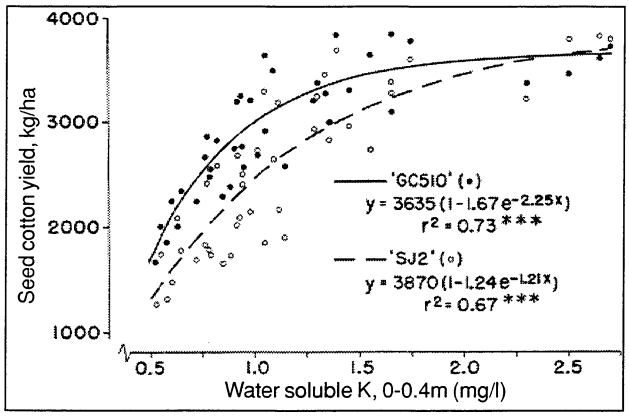

Figure 1. Relationship between seedcotton yield and water-soluble K levels in soil in the 0-40 cm depth interval for two cotton cultivars, GC510 and SJ-2, in a field experiment. Reprinted from Cassman et al.[10] with permission from the American Society of Agronomy.

Root distribution in relation to the distribution of available K in the soil profile also appears to be an important determinant of KUE. Availability of K is greatest in the surface layer, and typically decreases with depth because K recycled in crop residues or applied as fertilizer is incorporated in the topsoil, and K is a relatively immobile nutrient in most soils. This gradient is further accentuated in no-till and conservation tillage systems where K uptake by roots occurs in both surface and subsurface soil layers while K-containing crop residues are left on the soil surface with little or no incorporation. As a result, available K becomes greatly enriched in the top few cm of the soil profile[19].

Table 1. Cotton cultivar differences in root growth in a K-deficient soil during a 29 day period after peak bloom on July 21 and actual root length density (RLD) and diameter on August 19. Values shown are means for all roots in the 0 to 60 cm depth interval. Data modified from Brouder and Cassman[6].

	Jul 21 – Aug 19	Aug 19 Values	
Cultivar	Root surface area % increase	RLD** cm/cm^3	Root diameter mm
SJ-2	36	0.93	0.36
GC510	74	1.25	0.41

The importance of congruence between soil K availability and root distribution is most notable when crop species are compared in soils with stratified K availability[21]. For example, root length density of the fibrous root system in barley (*Hordeum vulgare* L.) closely followed the distribution of available K in soil profiles with different topsoil depth (**Figure 2**). The cotton root system did not. As a result, barley K uptake was 6.5 times greater than by cotton per unit of available soil K in the profile under well-watered conditions. This K uptake advantage and the degree of congruence between soil K and root distribution decreased with less frequent irrigation and greater drying in topsoil layers. While the experiment was conducted for a period of 68 day growth in a large-volume greenhouse pot system, the results are consistent with rooting patterns of the K-efficient and -inefficient cotton cultivars in the field. Of the total increase in root development during the 29-day period following peak flowering (**Table 1**), the largest increase occurred in the 0 to 15 cm topsoil layer where available K was 40 to 80 percent greater than in the 30 to 60 cm subsoil[6].

Poor cotton root development in topsoil layers may reflect greater sensitivity of cotton root growth to soil moisture content than for barley[44, 47]. The differences in root distribution do not appear to result from differences in root growth plasticity to the external supply of available nutrients because both cotton and barley exhibit a tremendous root growth response to localized supplies of N, a smaller response to P, and little response to external K supply[8, 18]. In cotton, N applied without K addition to half the soil volume in a split pot system resulted in a 3-fold increase in shoot dry matter, a 90 percent increase in lateral root development, and a 78 percent increase in K uptake in a K-deficient soil (**Table 2**). Hence, root growth plasticity to both soil drying and the external supply of N and P are important controls on K uptake from soil profiles with available K concentrated in the surface layer. Whether genotypic variation exists for these responses has not been evaluated.

Effects of root system architecture and root distribution in relation to profile K availability also appear to be important determinants of KUE for rainfed maize (*Zea mays* L.) in Minnesota[2]. Significant genotypic differences in root system traits were found among commercial corn hybrids, and these differences had significant effects on crop K status and performance in the field. In ridge-till and no-till corn systems of central Iowa, K deficiency symptoms have been observed during rain-free periods[52]. These symptoms disappear when soil is rewetted after a rainfall event. Presumably this temporary K limitation is caused by soil drying in the topsoil where available K is concentrated in these ridge-till and no-till systems.

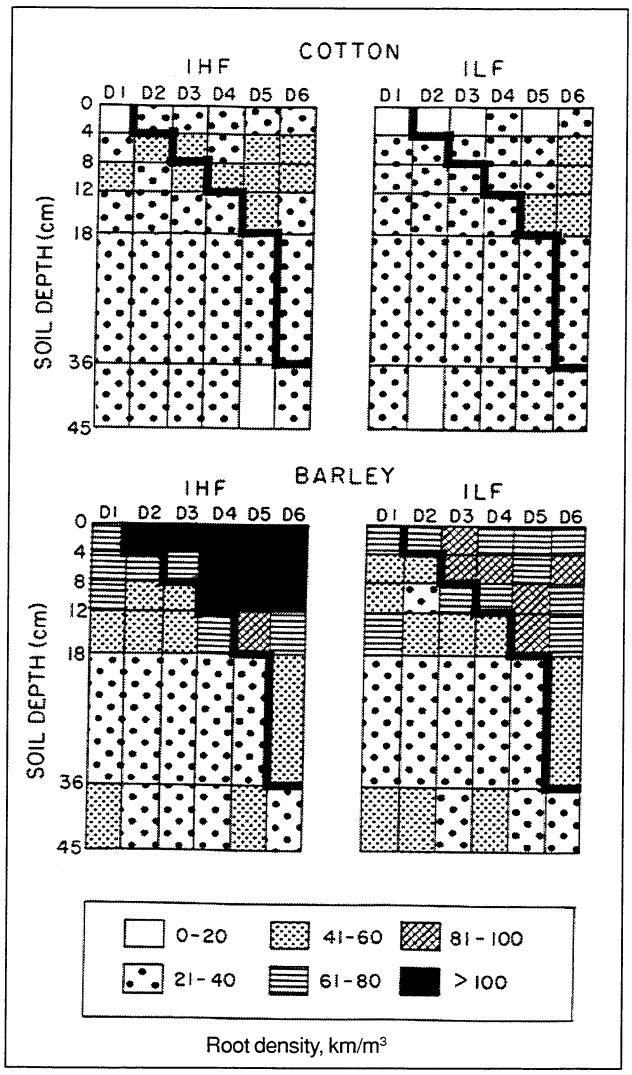

Figure 2. Root distribution of cotton and barley in layered profiles with a high (IHF) or low frequency (ILF) irrigation regime. Each column D1 to D6 represents the root distribution in treatments that differ in depth of topsoil. Depth intervals above the dark black line were occupied by topsoil (extractable K of 0.15 $cmol_c$/kg) and intervals below it by subsoil (0.09 $cmol_c$/kg). All other nutrients were provided in adequate amounts. Reprinted from Gulick et al.[21] with permission from the Soil Science Society of America.

Table 2. Effects of N deficiency on shoot growth and K uptake and lateral root development of cotton in a K-deficient soil with (+) and without (–) N or K fertilizer application. From Brouder and Cassman[8].

Soil treatment	Shoot		Lateral roots
	Dry weight, g/pot	K uptake, mg/pot	Root density cm/cm^3
–N–K	1.34 b†	18.4 b	4.2 b
–N+K	1.58 b	22.5 b	4.5 b
+N–K	4.03 a	32.7 a	8.0 a

† Means followed by different letters indicate a significant mean separation at $P<0.05$.

Foliar Fertilization

Like N, P, and other essential nutrients, K can be absorbed by leaves when applied as an aqueous K solution to the leaf canopy. Foliar fertilization to alleviate K deficiency is used on a number of crops. Although studies comparing the efficiency of K uptake from soil versus foliar application are lacking, foliar K fertilization has distinct advantages when the balance between soil K supply and crop requirements is uncertain, or when the response to soil-applied K is not consistent. Foliar-applied K permits a rapid response to the development of crop K deficiency, and repeat applications can be made as needed. Uncertainty in the K demand-supply balance is a common feature of rain-fed systems where yield potential is highly variable due to variation in rainfall patterns and amounts. The resulting soil moisture regime can influence crop K uptake through effects on root development, root distribution, and root activity as discussed in the preceding section.

Foliar-applied K may also be more efficient than soil applications to K-deficient soils with strong K fixation properties which reduce the uptake efficiency from soil-applied K. For example, less than 3 percent of applied K fertilizer was accounted for by increased K uptake in cotton grown on a K-fixing vermiculitic soil[13]. Although there are few direct measurements of uptake efficiency from foliar-applied K, the magnitude of yield response to foliar K applied to K-deficient crops indicates a relatively high uptake efficiency. A recent study also suggests that foliar K application to K-deficient cotton can increase kinetic parameters that govern the rate of K uptake at the root surface[28], which might result in greater K uptake from soil (see next section).

A disadvantage of foliar K fertilization is the relatively small amount of K that can be applied in a single application because of leaf injury from salt damage and solubility limitations associated with the need to minimize the application volume to reduce costs. Although there are differences among K sources in solubility and salt damage, even the most efficient sources such as KNO_3 and K_2SO_4 do not permit foliar rates in excess of 22 kg K/ha without leaf damage in cotton[37]. In contrast, K uptake requirements can exceed 5 kg/ha/day for a cotton crop in a high-yield environment[22]. Repeated foliar applications are required to correct K deficiency in these situations, and the costs of multiple applications can become prohibitive.

Potassium Uptake at the Root Surface

Uptake rate at the root surface is controlled by external factors such as [K^+] in the bulk soil solution, the [K^+] at the root surface, the effective K^+ diffusion rate, soil K buffer capacity, and the rate of water influx. These parameters can be modified by soil management although inherent soil physical and chemical properties place limits on the magnitude of modification that is feasible. Root physiological processes also govern uptake rates, and these can be quantified by three Michaelis-Menten kinetic parameters: the maximum rate of K influx (I_{max}), the external [K^+] at which the uptake rate is half of the maximum rate (K_m), and the minimum [K^+] of in the external solution (C_{min}) at which net influx is zero[4].

There are large differences in root K^+ uptake parameters among crop species, and differences have been documented among genotypes of the same crop species[3, 4]. Measurements to determine kinetic uptake parameters are made in nutrient solution culture and require precise control of environmental conditions. To my knowledge, however, genotypic differences in uptake parameters have not been related to KUE in a field environment. Justification for such efforts may not be strong because sensitivity analysis of factors affecting K uptake indicate that root uptake parameters are relatively insensitive compared to root length, profile distribution, and morphology[7, 46].

Plant N status can have a large effect on all K uptake kinetic parameters in cotton. Compared to control plants with adequate N supply, there was a 64 percent reduction in I_{max} and more than a three-fold increase in C_{min} in N deficient plants (**Figure 3**). In addition, accurate predictions of K uptake from soil in a pot experiment required specification of the appropriate uptake parameters as determined by plant N status[7]. These findings suggest that root uptake parameters may exert a significant influence on K uptake when crops are N deficient in a K-deficient soil with a high K buffering capacity. Many K-deficient soils have a high buffering capacity, and N deficiency is a widespread constraint. Some crops are purposely managed to provide a suboptimal N supply, especially during the reproductive growth phase. For example, moderate N deprivation is routinely practiced on irrigated cotton to avoid excessive vegetative growth and improve fruit retention[23].

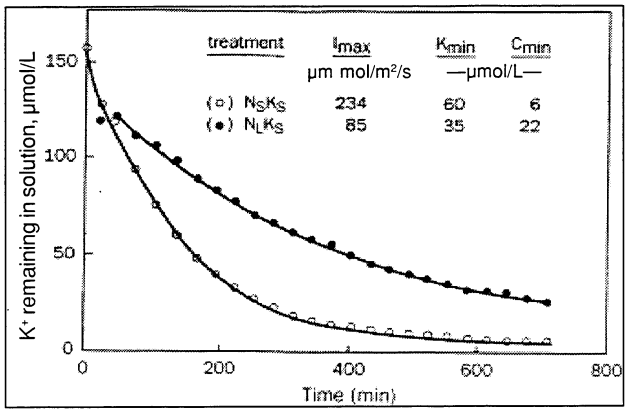

Figure 3. **Depletion of solution K^+ by 28-day-old cotton plants as influenced by preconditioning with sufficient N and K supply (NSKS) or deficient N and sufficient K supply (NLKS), and the Michaelis-Menten kinetic parameters calculated from these depletion curves. Modified from Brouder and Cassman[7].**

The increasing prevalence of late-season K deficiency on cotton in the USA[10, 37, 39] may result in part from decreased K uptake rates at the root surface caused by N deficiency during the period of peak K demand after flowering. Intentional N deprivation also occurs in cereal crops such as rice and wheat because these crops are more susceptible to diseases and lodging in high N-supply environments.

Utilization Efficiency within the Plant

Plant Potassium Requirements

Potassium is an essential nutrient for a large number of metabolic, transport, and osmoregulation processes. An early example of utilizing knowledge of plant nutritional physiology in agriculture was the identification of nutrient concentrations in plant tissues below which crop yields decreased. This knowledge led to use of plant tissue testing for diagnosis of nutrient deficiencies[497]. Since the 1930s and 1940s when much of the initial research was conducted, plant tissue testing has become an important tool in crop management.

Diagnosis of K deficiency by tissue analysis is based on empirically derived relationships between crop yields and the [K] of a specific index tissue such as the most recent fully expanded leaf blade or petiole. Critical levels and sufficiency ranges decrease with crop development so that stage of development must be specified when interpreting tissue tests. Sampled plant material is typically oven dried to constant weight at a standard temperature before analysis. Upon diagnosis of K deficiency, soil or foliar K applications can be made to correct the deficiency.

Although plant tissue testing is widely used, interpretation is complicated by environmental conditions, growth stage, yield potential and source-sink relationships, plant health, and interactions with the supply of other nutrients. As a result, there is often a wide range in sufficiency levels. In fact, nutrient tests based on dried plant samples provide a relatively crude approximation of plant K status.

Marschner[33] summarized a number of studies in which external K^+ requirements have been evaluated for specific metabolic processes such as enzyme activation and activity, protein synthesis, and photosynthesis. In general, these metabolic processes require an external [K^+] of 100 to 200 mM to achieve optimal rates, and this concentration is comparable to the [K^+] in cytoplasm of plants well supplied with K. Leigh and Wyn Jones[31] proposed that the [K^+] in tissue water might be a more accurate assessment of plant K status than tissue tests using dried plant material because K plays a primary role in the regulation of osmotic potential and cell turgor. Subsequent field studies across a wide range of soil K supply did not support this hypothesis: Tissue water [K^+] varied among plants well-supplied with K, presumably due to fluctuations in plant water status and substitution of other cations and solutes in the maintenance of turgor[5].

The role of K nutrition in improving the quality of some crops is another example of physiological knowledge used to improve K management. In cotton, adequate K supply is required for fiber development and maturity[15], and there is a direct relationship between [K] in cotton fiber and quantitative indexes of fiber quality[11]. A deficiency of K also affects the quality and marketability of several vegetable, tree fruit, and vine crops. Recognition of the K requirement for quality factors is sometimes considered in making fertilizer recommendations and in tissue testing guidelines for such crops.

Other examples of physiological knowledge used to improve KUE are difficult to find. Differences in K utilization among barley genotypes were related to differences in the cytoplasm [K^+] required for optimal rates of protein synthesis and differences in subcellular K^+ distribution between vacuole and cytoplasm[34, 35]. These studies were based on short-term growth effects in a hydroponic growth chamber system. The impact of differences in K^+ compartmentation and protein synthesis has not been quantified under field conditions. Without such verification, the agronomic benefits of genetic variation in processes associated with subcellular K utilization efficiency remain to be determined. It is possible that the observed genotypic differences in K efficiency were expressed as a result of differences in adaptation to the environmental conditions of the growth chamber system. Likewise, differences expressed during the early vegetative phase of plant development may not be expressed at later developmental stages.

Sodium-Potassium Substitution

Partial substitution of Na^+ for K^+ requirements in the maintenance of osmotic potential and cell turgor has been recognized for quite some time. Mechanisms responsible for this replacement are reviewed by Marschner[33] and Lauchli[30]. In recent years, interest in Na-K interactions have mostly focused on the relationship between salinity tolerance and mechanisms that govern Na^+ exclusion. These exclusion processes allow maintenance of [K] in shoot tissues which in turn reduce growth reductions from salinity. Much less emphasis has been given to utilizing Na replacement to increase KUE.

More than 30 years ago, Joham and Amin[27] suggested that a discussion of K deficiency in some crops must take into account substrate levels of Na. For example, Lunt and Nelson[32] found a large decrease in the external K supply requirements of cotton grown to maturity in a large capacity sand culture system. In a recent field study, Cassman et al.[12] observed a similar shift in the cotton yield response to soil K availability that was related to increased soil Na (**Figure 4**). The increase in soil Na resulted from use of slightly saline groundwa-

ter during a 3-year drought period when normal access to low-salt canal water became increasingly restricted. Continuous use of the low-salt canal water in previous years leached nearly all Na from the active root zone. After mixed use of both canal and groundwater for irrigation during the drought years of 1988-1990, [Na] in the soil solution increased from 0.07 mM in 1987 to 1.16 mM in 1990. As a result, the soil K requirement to achieve 90 percent of maximum seed cotton yield decreased from about 4 mg K/L in 1987 to 3 mg K/L in 1990[9]. This shift was equivalent to an application of 300 kg K/ha to the low-K soil in 1987 because of the large K fixation capacity of this soil.

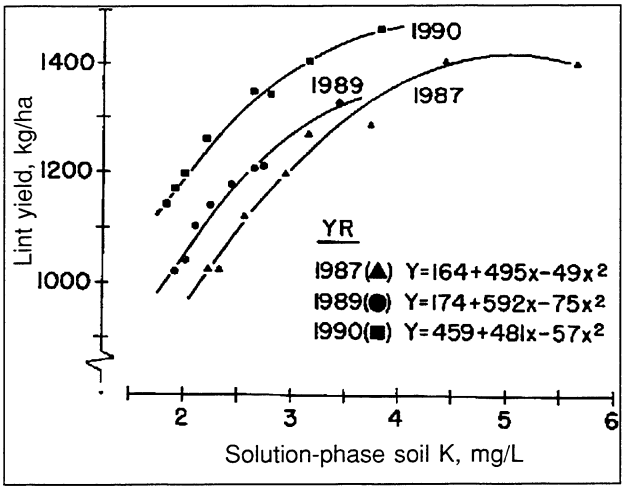

Figure 4. Shift in the lint yield response to an index of soil K supply associated with an increase in Na concentration in the soil solution that resulted from use of slightly saline groundwater in 1989 and 1990. Modified from Cassman et al.[12].

There are large regions where soil K deficiency is a constraint to irrigated crop production and where farmers have access to both low-salt surface water and saline groundwater. These regions include the Central Valley of California, central Asia, and the Indo-Gangetic Plains of northern India and Pakistan. To my knowledge, intentional use of slightly saline groundwater in conjunction with high quality surface water is not used as a management tool to increase KUE and minimize K fertilizer requirements in these regions.

Potassium Balance with Other Nutrients

The concept of a functional balance among the internal requirements for the essential plant nutrients has been discussed since the 1930s and 1940s[49]. It has been difficult to validate this concept experimentally, however, because of the effects of environmental conditions and crop growth stage on the critical sufficiency levels for each nutrient. More recently, nutrient balance ratios have been used in a Diagnosis and Recommendation Integrated System (DRIS) to identify the most limiting nutrients by plant analysis[53]. The disadvantages of this approach are the cost of multiple nutrient analyses, the complexity of interpretation, and inaccurate diagnosis of the limiting nutrients in some cases. The optimum nutrient ratios are empirically determined because the scientific basis for them is not well developed.

Despite the lack of strong scientific evidence, the existence of functional nutrient balances within broad limits would seem to be a reasonable supposition because of antagonisms between certain nutrients that affect uptake at the root surface or function within the plant. Moreover, as average yields achieved by farmers continue to increase, maintaining a functional balance between supply and demand for essential nutrients may result in narrower margins for error in meeting the complete nutritional requirements of high yielding crops.

Nutrient balance constraints are evident in the results from a long-term field experiment on irrigated rice (*Oryza sativa* L.) in the Philippines (**Table 3**). From 1989-1991, N was most limiting to grain yields, there was a small response to P, and no response to K application. The maximum yield levels achieved during this period were considerably less than the attainable yield potential predicted by a crop simulation model[29]. Increasing the quantities of applied N, P and K in a subsequent 3-year period (1992-1994) resulted in a 41 percent increase in grain yield when complete N, P, K fertilizer inputs were applied. There was a significant response to both P and K at these higher yield levels despite the lack of a response to K at the lower yield levels achieved during the 1989-1991 period.

Table 3. Yield of irrigated rice variety IR72 in a long-term experiment with different fertilizer treatments in the Philippines. Rates of applied nutrients were increased in the 1992-1994 period. Modified from Cassman et al.[14].

Fertilizer rate						Mean grain yield†	
N	P	K	N	P	K	1989-1991	1992-1994
1989-1991			1992-1994				
kg/ha						t/ha	
0	0	0	0	0	0	2.74 c‡	3.75 c
140	0	0	200	0	0	5.12 a	6.97 b
140	13	0	200	25	0	5.35 a	6.95 b
140	0	25	200	0	40	4.67 b	7.00 b
140	13	25	200	25	40	5.27 a	7.42 a

† Values for each treatment are pooled means from three dry season crops in the 3-year period indicated.
‡ Within each site and period, means followed by the same letter are not significantly different at P<0.05.

Soils at this site were well supplied with K. In fact, extractable soil K in all treatments was more than five-fold greater than adequate soil-test levels, and the [K] in plant tissues also tested above sufficiency thresholds[16, 17]. The reasons for this K response and the associated nutrient balance interactions have not been identified. Most likely, they are caused by an antagonism from relatively

high levels of soil Ca and Mg at this site which can reduce K diffusion or uptake at the root surface. Constraints due to internal nutrient imbalances within the plant are also a possible contributing factor.

Agronomic Measures of Physiological Efficiency

The most agronomically relevant index of physiological efficiency is the incremental increase in economic yield per unit of plant K accumulation. This index is estimated by the slope of the relationship between yield and plant K content at physiological maturity as illustrated in **Figure 5** for irrigated rice[16, 26].

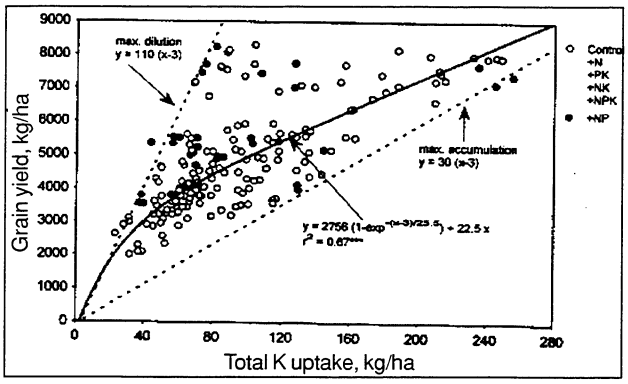

Figure 5. Relationship between rice grain yield and K accumulation in above-ground biomass at maturity in 11 long-term experiments in five Asian countries. Values are replicates of six fertilizer treatments sampled in the 1993 dry season. Fertilizer treatments include a control without applied nutrients and various combinations of applied N, P, and K. The +NP treatment imposed the greatest demand on native soil K supply because yields were not limited by N or P. The dashed lines indicate the possible envelope of maximum K accumulation and maximum K dilution in the rice plant. The solid line is the average function fitted to the complete data set. Modified from Dobermann et al.[16, 17].

Highest physiological K efficiency is achieved when K is the nutrient most limiting to crop growth. These conditions occurred in treatments that received only N and P fertilizer (+NP treatments in **Figure 5**) at sites where soils were K deficient. Except for sites where soils were well supplied with K, data points from the +NP treatments tended to fall closer to the boundary of maximum K dilution within the plant, which corresponded to a physiological efficiency of approximately 110 kg grain per kg K accumulation. In sites and treatments where the K supply was adequate and plant growth was limited by other nutrient deficiencies, the data points shift towards the line of maximum K accumulation represented by a physiological efficiency of 30 kg/kg.

It is not practical to manage crop K supply to achieve a physiological K efficiency at the boundary of maximum plant K dilution because of the potential for K limitation and because this strategy ignores effects on crop quality and disease interactions. A more appropriate goal is to shift the "average" physiological efficiency, as represented by the regression line, toward the maximum dilution boundary by use of improved crop and soil management practices. Proper nutrient balance is a prerequisite for such a management strategy on all soils, and intentional Na-K substitution would be a component under certain conditions as previously discussed. Modern crop varieties with a higher harvest index than the traditional varieties have also contributed to increased physiological K efficiency, especially for cereal crops which have relatively low [K] in grain. In contrast, concerted efforts to improve physiological K efficiency of crop varieties by selection for specific mechanisms governing K utilization have not been attempted because it is not clear which plant traits and processes would increase KUE under field conditions.

Plant Potassium Status and Disease Susceptibility

The incidence and severity of a number of diseases increase when crops are K deficient. Disease interactions with plant K status have been documented for both fungal and bacterial pathogens, and for pathogens that infect the plant via roots, stems, leaves, and fruiting structures. This interaction is agronomically important. For example, it is has been suggested that K deficiency is a prerequisite for leaf infection by the bacterial pathogen *Alternaria macrospora* (A. Zimmerm.) that causes leaf spot in cotton[24]. Also on cotton, the incidence and spread of leaf lesions caused by *Verticillium dalhiae* (Kleb), a fungal pathogen, was 50 to 60 percent greater in K-deficient plants, although the incidence of vascular infection in main stems was unaffected by K status[12]. Because roots are the initial site of infection by the verticillium wilt pathogen and the disease progresses through stems to leaves, a reduced number and size of leaf lesions in K-sufficient plants must result from suppression of disease progression.

The mechanisms that control interactions between disease severity and plant K status are poorly understood[25]. One hypothesis is that the accumulation of soluble carbohydrates and N compounds in K-deficient leaves provides a better substrate for growth of disease organisms which increases the rate of disease development. Whatever the mechanism, it is clear that plant K status plays an important role in the suppression of several major diseases on a number of important food, fiber, and forage crops. This suppression can have significant effects on physiological K efficiency in environments with high disease pressure.

Research Challenges and Opportunities

The contributions of plant physiology research to improved KUE during the past 70 years have been modest. Plant tissue testing for diagnosis of K deficiency and use of foliar fertilization are the two most prominent examples. Knowledge of plant

K supply effects on crop quality has also been used to develop improved K management recommendations for several crops. Recognition of the need for improved congruence between root distribution and K availability in the soil profile has prompted testing of K fertilizer placement on cotton in subsurface soil where soil moisture undergoes less fluctuations than in topsoil[36, 48]. Similarly, maize hybrid varieties with root development traits adapted to rain-fed no-till and ridge-till systems are also under investigation as a means to improve KUE[2]. Awareness of reduced disease severity with improved plant K status has resulted in consideration of K fertilization as a component of disease management.

But why such relatively modest contributions to improved KUE despite exciting advances in knowledge of K physiology at the whole plant, organ, cell, and subcellular levels as reviewed elsewhere in this proceedings? One answer is the apparent lack of commitment to validation of hypotheses about physiological processes governing KUE in relevant field environments. Such validation requires collaboration among plant physiologists, ecophysiologists, agronomists, soil scientists, and plant geneticists. Perhaps these partnerships have been limited by availability of funds or by the discipline-based organization of research institutions. These constraints must be abated.

Assuming they are, and given the projections for a tremendous increase in K fertilizer usage to ensure global food security, how will plant physiology contribute to meeting the challenge of increasing KUE? The goal is clear: We must accelerate the development of improved crop cultivars and improved management systems that increase K uptake and utilization efficiency.

The most obvious opportunities for contributions to this goal are related to plant traits and responses already known to have a significant impact on KUE under field conditions. Based on existing knowledge, the most promising opportunities are associated with the potential to modify root morphology, development and distribution by soil management and genetic improvement. Success will depend on a more comprehensive understanding of process controls on root initiation, branching, and elongation rates in relation to the external environment and feedback mechanisms within the root system and shoot. Present understanding of these processes is not sufficient to accurately predict rooting patterns and K uptake under field conditions[51].

Of particular importance is root growth plasticity in response to localized nutrient supplies and soil moisture and whether genetic variation exists for these responses. An ability to predict root development rates and rooting patterns would aid development of K fertilization practices that increase the congruence between soil K availability and active root surface area in time and space. If useful genetic variation is found for root traits or root growth responses, it is likely that they are under complex genetic control involving a number of genes. Identifying quantitative trait loci associated with these traits would facilitate conventional breeding efforts.

Foliar K fertilization will likely become more important as crop yields continue to increase during the next 25 years. Higher yielding crops have greater K uptake requirements, and the magnitude of variability in yield and K requirements due to climate and other production factors also increases. Optimal efficiency might be achieved using soil-applied K to meet crop requirements in "normal" years with average yields, while foliar K could provide additional inputs in years with greater K requirements because of higher than normal yields. Results from a recent field study indicate the potential for greater KUE using both soil- and foliar-applied K[42]. Research is needed on novel K compounds and formulations to increase the amount of K that can be applied without leaf injury and on improving K absorption efficiency into the leaf.

Other areas that hold promise are Na-K substitution, nutrient balance, and disease suppression. The ability to predict Na and K uptake in relation to soil availability of these elements and quantitative knowledge of Na replacement in physiological processes would allow estimation of Na substitution without a decrease in yield or crop quality. This information could be used to control the [Na] in irrigation water to minimize K fertilizer requirements and avoid soil salinization. In contrast to Na-K replacement about which much is known, present understanding of nutrient balance and K-disease interactions is mostly qualitative. Impact on KUE at the field level will therefore require increased knowledge about the processes responsible for these interactions.

The potential for genetic improvement in K uptake efficiency at the root surface or internal efficiency at the whole plant, cellular, or subcellular levels is not clear. Although genetic variation has been documented for some of these traits, the impact on KUE by selection for improved root uptake mechanisms or physiological processes governing utilization efficiency within the plant have not been documented at a field scale that includes a complete crop growth cycle. Because plant N status appears to have a significant impact on K uptake kinetics, however, research on the process controls and magnitude of this interaction may help identify management options that increase K uptake during the reproductive growth phase, particularly for crops with large K requirements in seed or fruiting structures. Such an increase in K uptake would be most beneficial in situations when crops are intentionally provided a suboptimal N supply after the onset of flowering to improve fruit retention, avoid rank growth, or reduce the potential for diseases and lodging.

Summary

This paper reviews the contributions of plant physiology research to increased K-use efficiency (KUE) in crop production systems and identifies promising directions for future research. Relatively few cases were identified in which knowledge of physiological processes governing plant K uptake and utilization has been used to improve KUE under field conditions. In large part, this poor rate of success can be attributed to lack of strong collaboration between physiologists working at different levels of organization, from molecular and subcellular levels to plant communities, and insufficient integration with other disciplines such as plant genetics, agronomy, soil science, and plant pathology. A commitment to validation of hypotheses about mechanisms that control KUE in relevant agronomic environments is also needed, but this commitment is often absent in many plant physiology programs.

The most promising opportunities to increase KUE from future investment in plant physiology research are associated with the ability to modify root development and function. Manipulation of both genetic and environmental controls on root traits appears to be deserving research targets. Novel approaches involving foliar K fertilization and Na-K substitution also appear to hold promise under certain conditions. Potential contributions from molecular biology must await improved understanding of the basic processes and mechanisms that govern KUE under field conditions and the genetic control of these mechanisms. Use of transgenic plants may prove to be a valuable research tool in this quest for knowledge.

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