Engineering Plant Zinc Deficiency Tolerance

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INTRODUCTION

Zinc (Zn) deficiency among crops is a widely occurring phenomenon causing decreased crop yields and crop quality. Plants have a natural response mechanism to overcome low Zn bioavailability by increasing the expression of several genes involved in Zn homeostasis, including genes encoding Zn transporters and metal chelator biosynthesis enzymes (van de Mortel et al., 2006). Recently we identified the transcription factors that are essential for switching on the Zn deficiency response of *Arabidopsis thaliana* (Assunção et al., 2010). These genes (*bZIP19* and *bZIP23*) encode two bZIP transcription factors, that recognize palindromic motifs, called Zinc Deficiency Response Elements, found in tandem in several Zn homeostasis genes. These *bZIP* genes act largely redundant, meaning that only when both genes are mutated, plants will show extreme sensitivity to Zn deficiency (Assunção et al., 2010). Genetic modification of the expression of these bZIP transcription factors allows the engineering of plant Zn deficiency tolerance, which could offer an attractive advantage for crops in areas suffering from low Zn bioavailability. Here we describe the progress in this research, leading to a proof-of-principle.

METHODS

Three binary plasmids were constructed, placing the *bZIP19* or the *bZIP23* gene under the transcriptional control of the strong constitutive CaMV 35S promoter, or under control of the Arabidopsis Zn deficiency responsive *ZIP4* promoter (only for *bZIP19*). Constructs were transformed to Arabidopsis. Homozygous lines with good expression of the transgene were selected and plants were grown along with wild-type plants on hydroponic medium containing either normal Zn supply (2 µM $ZnSO_4$) or low Zn supply (0.05 µM $ZnSO_4$). After four weeks, plants were harvested (roots and shoots) and their dry weight was determined. In addition, material was collected to determine gene expression and mineral concentrations.

RESULTS AND DISCUSSION

Initially plants overexpressing either *bZIP19* or *bZIP23* were compared to wild-type plants. Plants from transgenic lines generally looked better than wild-type plants, showing larger rosettes and less chlorosis. However, when root and shoot dry matter was determined, transgenics performed only marginally better than wild type, under low Zn supply (data not shown). We reasoned that general overexpression of the *bZIP* genes might disturb the precise regulation required for proper action of the downstream genes, and such deregulation would not lead to the required effect. Therefore, instead of an overexpression construct, a more regulated construct was used. In this construct, the expression of the *bZIP19* gene is controlled by the promoter of the *ZIP4* gene, itself a regulatory target of *bZIP19* (Assunção et al., 2010). The expectation was that the response to Zn deficiency would be switched on at a very early stage of Zn deficiency and only in the cells that normally are involved in the Zn deficiency response.

Again, three independent transgenics were selected and two replicate lines of each were maintained and brought to homozygosity before testing. Plants were treated as described and grown along with wild-type plants on hydroponics with normal and low Zn. After four weeks, transgenic plants on low Zn medium looked a lot better than wild-type plants (data not shown). This time, the effect was also reflected in the root and shoot dry weight (Figure 1). Currently we are

analysing the mineral concentrations in these plants and determine the expression of direct and indirect Zn homeostasis target genes of *bZIP19*.

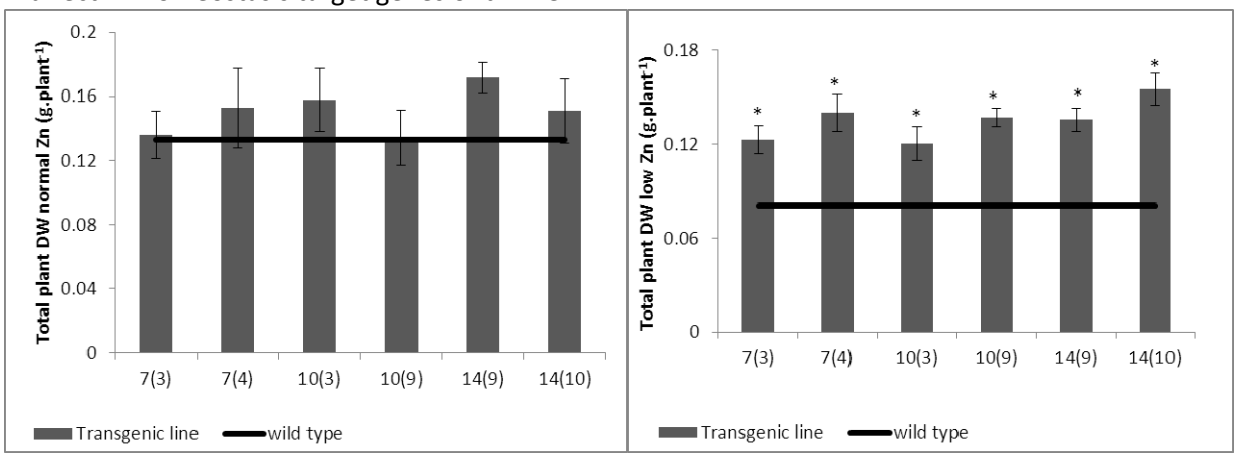

Fig. 1. Total plant dry weight of two lines from three independent homozygous transformants (7, 10, 14) expressing proZIP4::bZIP19 grown for four weeks on hydroponic medium with normal (2 µM) or low (0.05 µM) $ZnSO_4$ supply, compared to Col-0 wild-type plants. * indicates significant difference from wild-type Col-0 at P<0.05.

CONCLUSIONS

CaMV-mediated overexpression of either of the two *bZIP* transcription factors controlling the essential Arabidopsis Zn deficiency response, has only little effect towards improving Zn deficiency tolerance. However, controlled enhanced expression of *bZIP19* by placing this gene under control of the Zn deficiency responsive *ZIP4* promoter, has been very effective in enhancing Arabidopsis Zn deficiency tolerance, almost doubling their dry weight compared to wild type.

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