X-ray Fluorescence Analysis as a Tool for the Breeding of Zinc and Iron Dense Pearl Millet

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INTRODUCTION

Considerable effort is being made around the world to breed nutrient-dense grain crops for improved human nutrition. Within the HarvestPlus biofortification program (Nestel et al. 2006), a major focus is to breed pearl millet cultivars containing more Zn and Fe. Reliable and cost effective methods to analyse Zn and Fe in grain samples are clearly fundamental to these efforts. At present, plant breeders rely heavily on inductively coupled plasma-optical emission spectrophotometry (ICP-OES) and atomic absorption spectroscopy (AAS) for elemental analysis. However, these forms of analysis require extensive sample preparation, access to expensive, contamination free reagents and can only be conducted by trained analysts. Many breeding programs around the world are unable to undertake these analyses themselves and have to out-source analytical work, often internationally, at considerable expense and inconvenience.

The technique of X-ray fluorescence analysis (XRF) has been shown previously to be suitable for elemental analysis of a wide range of organic and inorganic samples. Though initial applications for the technique were predominately in geochemistry and manufacturing, XRF has also been used successfully in the analysis of processed foods and beverages (e.g., Nielson et al. 1988, Perring et al. 2005). In all these studies, samples were dried, ground and pressed into pellets prior to analysis, and XRF was shown to be reliable and cost-effective, particularly for the analysis of Cu, Fe and Zn. This study investigates whether XRF can be used to provide a cheap, non-destructive method for elemental analysis in pearl millet. Emphasis is on Zn and Fe, and on whole grain samples rather than flour, with a view to identifying methods useful in large scale pearl millet biofortification breeding programs.

METHODS

The pearl millet samples used in this study were all from breeding programs connected to HarvestPlus, and all had been analysed using ICP-OES for Zn and Fe at Waite Analytical Services. XRF was performed using an Oxford Instruments X-Supreme 8000 EDXRF. Measurement conditions were as recommended by the manufacturer for analysis of Zn and Fe in a cellulose based matrix and an acquisition time of 60 secs. Scans of grain samples were achieved simply by pouring 7 ml of each sample into XRF cups sealed at one end by 4µm Poly-4 film. Calibrations for Fe and Zn in pearl millet were achieved using 20 samples. Samples were chosen to ensure that the full range of Zn and Fe concentrations seen in HarvestPlus breeding material was represented. Based on ICP data, Zn ranged from 19 to 155 mg kg^{-1} and Fe from 16 to 115 mg kg^{-1}. To validate calibrations, a further 20 pearl millet samples were randomly selected from a breeders population and scanned. After scanning, Zn and Fe concentration results were plotted against reference values, and correlation indices, standard errors of prediction (SEP) and 95% confidence intervals (CI) were calculated.

RESULTS

The validation data presented here was attained in 45 min without the use of any consumables or reagents besides the XRF film used to seal sample cups. Strong correlations were observed between XRF- and ICP-determined concentrations of Zn and Fe (Fig 1), with R^2=0.96 for Zn and 0.93 for Fe. According to paired t-tests, known and XRF-determinations were not significantly different for Zn or Fe. SEP were 2.9 mg kg^{-1} for Zn and 7.5 mg kg^{-1} for Fe. Confidence interval statistics indicated XRF Zn values can be expected to be mostly within ±6 mg/kg of ICP values, and XRF Fe values within ±15 mg kg^{-1} of ICP values.

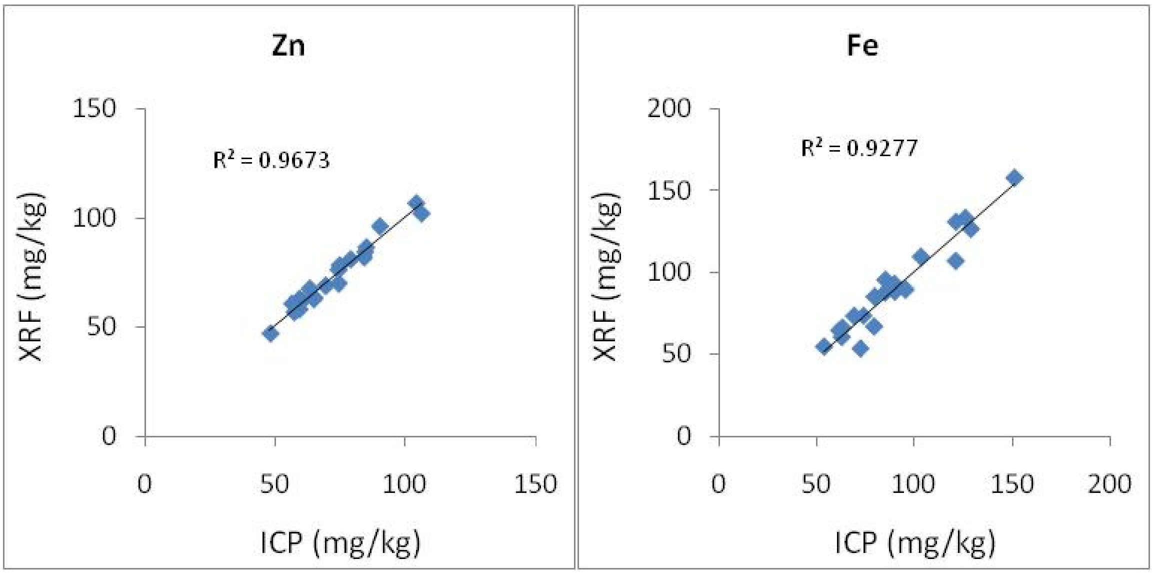

Fig. 1. Correlations between XRF- and ICP-determined Zn and Fe concentrations in pearl millet grain.

CONCLUSIONS

Programs targeting high Zn and Fe pearl millet require rapid and cheap assays for these elements in whole grain samples. It is expected that the accuracy and precision of XRF Zn and Fe analysis demonstrated here will lead to the adoption of the technique in pearl millet biofortification programs, leading to increased throughput and major cost savings. Work is now underway to explore the use of XRF in analyzing Zn and Fe in grain and flour samples from other important crop species.

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