Development of a Practical Staining Method for Visualizing Zinc in Wheat Grain and Flour

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INTRODUCTION

Inherently low levels of Zn in cereal grains and widely applied milling practices are the major factors leading to Zn deficiency in humans and related health problems, particularly in communities where cereals are the major source of daily calorie intake. During the milling process, a significant portion of Zn within the seed is lost by the removal of bran which consists of the aleuron and the embryo, the Zn-rich parts of wheat grain. Dithizone (DTZ: diphenyl thiocarbazone) is a Zn-chelating agent and produces a reddish color after complexing Zn (McNary 1954). Although it has been used to identify Zn localization in different organisms, such as wheat seeds (Ehret 1985), there is very little knowledge on the application of the DTZ staining method to different genotypes and flour products. In this study, a practical staining method was developed to locate Zn in the wheat grain using grains with a wide range of Zn concentrations (i) to have a better understanding of Zn localization in wheat grain, Zn deposition during seed development and Zn remobilization during germination, and (ii) to develop a rapid visual screening method for Zn in grain and flour.

METHODS

Grain samples collected at different seed development stages were embedded in epoxy resin blocks and ground to produce polished longitudinal grain sections. The sample blocks were stained for 30 min in freshly prepared DTZ solution (i.e. 500 mg L^{-1} 1,5-diphenyl thiocarbazone in analysis grade pure methanol). Mature grains were imbibed for 1 h in water prior to excision, whereas germinating seeds were directly excised longitudinally along the crease with scalpel and stained in DTZ solution. Flour samples were prepared by grinding whole wheat grains with an agate mill. About 200 mg of each flour sample was placed in 24-well plates, and surfaces were flattened by a plastic bar prior to staining with 200 µl of the DTZ solution. Samples were photographed using a reflectance light microscope and a high resolution digital camera. Correlation of Zn concentration with DTZ staining was tested in methanol extracts of the stained flour samples as described by Ozturk et al. (2006).

RESULTS AND DISCUSSION

The DTZ staining method confirmed that Zn is predominantly localized in the embryo and aleuron parts of the seeds (Fig. 1A). The Zn concentrations seemed to be around 125-150 mg Zn kg^{-1} in embryo and aleuron parts and around 10-15 mg Zn kg^{-1} in the endosperm. Staining was more intense at the beginning of seed development (i.e. 12 days after anthesis) and comprised the whole seed. As the seed development progressed, staining was reduced and Zn predominantly localized in embryo and aleurone parts (Fig. 1A). In germinating seeds with contrasting Zn concentrations, Zn was particularly concentrated in the coleoptile and the root tip (Fig. 1B) suggesting an intensive remobilization of Zn to the newly emerging parts at early stage of germination. Flour samples with increasing Zn concentration were also stained successively with DTZ (Fig. 1C). The color produced showed a significant correlation with Zn concentrations that were measured by Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES) (Fig. 1D).

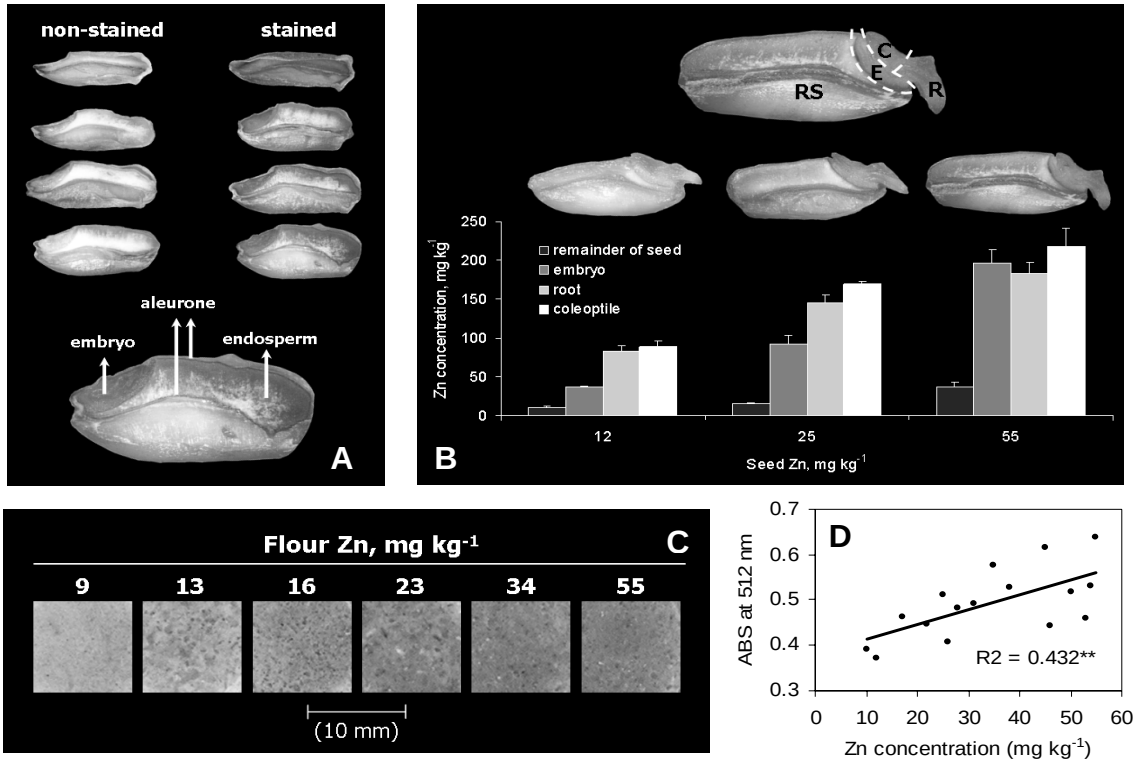

Fig. 1. (A) Staining of wheat seeds sampled at (top to bottom) 12, 24, 34 and 52 days after anthesis. (B) Concentration of Zn in different seed parts and dithizone staining of seeds during germination. (C) Dithizone staining of flour samples with increasing Zn concentration. (D) Relationship between Zn concentrations and spectral absorbances of methanol extracts of flour samples stained with DTZ.

CONCLUSIONS

We conclude that the accumulation of Zn in seeds is particularly high during the early seed development and that Zn is concentrated in the embryo and aleurone parts through developmental stages. The DTZ staining method demonstrated that seed-Zn is mostly accumulated in aleurone and embryo, which might be associated with high levels of Zn-binding compounds, such as proteins and phytate. The developed DTZ method can be useful in semi-qualitative screening for Zn in whole grain and flour samples as judged by the significant relationship between Zn concentrations and spectral absorbance of methanol extracts of flour samples stained with the DTZ method.

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REFERENCES

Ehret, M. (1985) Verteilung von Phytinsäure, Zink und anderen Mineralstoffen in Weizenkörnern unter morphologischen, technologischen und ernährungsphysiologischen Aspekten. PhD thesis. Hohenheim University, Stuttgart.

McNary, W.F. (1954) Zinc-dithizone reaction of pancreatic islets. J Histochem Cytochem 2: 185-195.

Ozturk, L., Yazici. M.A., Yucel, C., Torun, A., Cekic, C., Bagci, A., Ozkan, H., Braun, H.J., Sayers, Z. and Cakmak, I. (2006) Concentration and localization of zinc during seed development and germination in wheat. Physiol. Plant 128: 144–152.