

Grain Characteristics That Define Zn-Dense Wheat

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INTRODUCTION

A considerable amount of knowledge has been generated to improve our understanding of Zn transport into the grain (Pearson and Rengel 1994, Pearson et al. 1998, Garnett and Graham 2005) as well as its localization in grain tissues (Ozturk et al. 2006). It is important to utilize and add to this information to develop Zn-dense wheat varieties. This study aims to identify Zn in principal components of the grain (crease, pericarp, embryo and endosperm) and in all tissues of a whole spike (glume, lemma, palea, grain and rachis) and flag leaf to better understand what constitutes a Zn-dense genotype.

METHODS

Plant Materials

Two wheat (*Triticum turgidum*) varieties (Samnyt16 and Pastor) were grown in nutrient-rich UC potting mix with slow-release fertilizer (Osmocote Plus[®]) added to each pot three weeks after transplanting. Plants were watered daily to keep the soil moist. Anthesis date was recorded by the appearance of anthers, and the whole spike was harvested 21 d later for the separation of various tissues (glume, lemma, palea, grain and rachis). For the larger screening of 70 wheat varieties by staining techniques, varieties were grown at the same site in Mexico.

Grain dissection and analysis

Each grain was sampled from florets within four central spikelets, and the grain was immediately dissected within 1-2 mins of harvesting. Crease tissue (including vascular bundle and nucellar projection) was sampled according to Ugalde and Jenner (1990). The tip of a forcep was inserted into the endosperm cavity at one end of the grain and slid along its full length. The outer transparent layer of pericarp was then peeled from the grain followed by peeling of the inner pericarp tissue from the endosperm. Embryos were isolated microscopically from the endosperm. Samples were analysed by Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES). Localisation of Zn in tissues was also achieved by staining with dithizone, which is a Zn-chelating agent (Ozturk et al. 2006). The intensity of colour produced after staining was semi-quantified using Adobe Photoshop[®] image analysis software. Coloured pixels were quantified across a cut surface of the grain. Genotypic variation in coloured pixel numbers was measured in 70 wheat genotypes.

RESULTS AND DISCUSSION

Twenty one days after anthesis, SAMNYT16 and Pastor had varying amounts of Zn in rachis, crease and embryo, while there was no significant variation in Zn in other tissues (Fig. 1; Table 1). Generally, in the spikelet and flag leaves, Zn concentrations varied in the following order: rachis > palea > lemma > glumes = flagleaf, and in the grain: crease > embryo > inner pericarp > outer pericarp > endosperm. Using the dithizone reagent to identify Zn in grain tissues, there appeared to be variation in intensity of the red colour produced, and the variation was seen most in crease and embryo regions. The ratio of stained red pixel number on the cut surface to total grain weight was associated with total grain-Zn concentrations measured by ICP-OES (Fig. 2). The semi-quantification of Zn by colour

image analysis revealed significant genotypic variation that can be exploited for development of Zn-dense wheat.

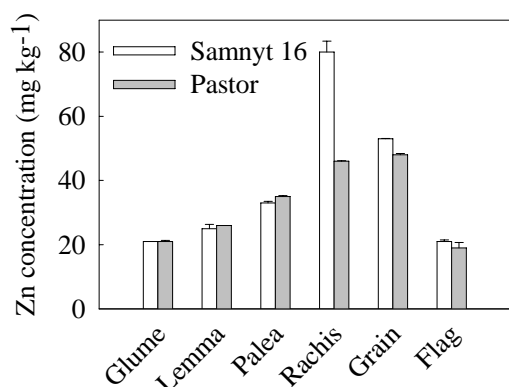


Fig. 1. Zinc concentration in tissues of the whole spikelet and flag leaf

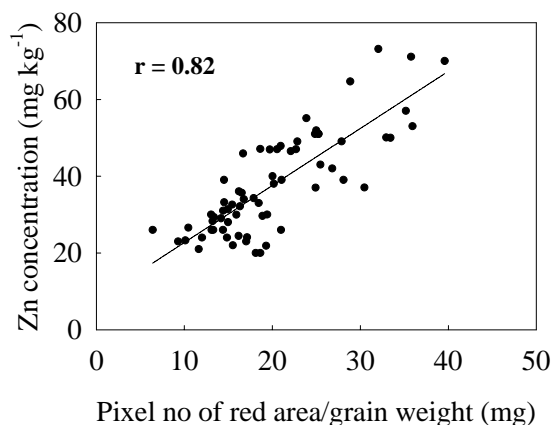


Fig 2. Relationship between the total red pixel number : total grain weight ratio and Zn measured by ICP

Table 1. Zinc (mg kg⁻¹) in various grain tissues from wheat harvested 21 d after anthesis, and in mature grain. Data represent Mean \pm SE of seven replications for various sections of the grain, while for matured grain analysis, 3 replications were used.

	Crease	Pericarp (outer)	Pericarp (inner)	Embryo	Endosperm	Mature grain
SAMNYT 16	130 \pm 15	49 \pm 2	66 \pm 4	80 \pm 5	30 \pm 1	49 \pm 5
Pastor	87 \pm 7	50 \pm 3	74 \pm 3	70 \pm 6	29 \pm 1	37 \pm 2

CONCLUSIONS

Zn-dense wheat appeared to have more Zn in the rachis during the grain filling period, and this was associated with more Zn in various grain tissues including the crease and embryo. The role of the rachis as a source of Zn for sink tissues is required. Increased Zn in the crease (vascular tissue and nucellar projection) of the grain may provide a target site that can be exploited for plant breeding and molecular purposes.

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