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Zinc distribution and localization in primed maize seeds and its translocation during early seedling development

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ABSTRACT

Zinc (Zn) priming is a technique used to increase seed Zn reserves for improving seed quality, crop growth, and enhancing stress tolerance in crop plants. The present study demonstrated the effect of water and Zn priming on the distribution and accumulation of endogenous and primed Zn in maize seeds (*Zea mays* L.). Zn concentration in unprimed, water and Zn primed seeds and germinated seedlings were analyzed by ICP-MS (Inductivity Coupled Plasma Mass Spectroscopy). DTZ (Diphenyle Thio-Carbazone) staining method and LA-ICP-MS (Laser Ablation Inductivity Coupled Plasma Mass Spectroscopy) scanning was used for showing Zn distribution and localization in the seeds. Zn priming significantly increased Zn concentration and content in seeds. Results of ICP-MS analysis showed a substantial increase in the testa and endosperm tissues after Zn priming. DTZ staining and LA-ICP-MS scanning of maize seeds revealed an uneven distribution of Zn in water and Zn primed seeds. Laser ablation data of water primed maize seeds demonstrated a significant ($p < 0.05$) relocation of endogenous Zn from the aleurone layers towards the inner endosperm. Zn priming increased endosperm Zn content 3-fold compared with water primed seeds, while in the testa this increase was 50-fold. Furthermore, Zn priming significantly ($p < 0.05$) increased the biomass of 10-d old maize seedlings grown in rolls of filter paper. Translocation of primed Zn towards shoots and roots was double than that of endogenous Zn in unprimed and water primed maize seeds. This is the first report of the distribution and accumulation of primed Zn in maize seeds. Further investigations are needed to understand the binding capacity of the different tissues within maize seeds and the retranslocation of primed Zn during early seedling development and plant growth.

1. Introduction

Zinc (Zn) deficiency in agricultural soils is a yield limiting factor for cereal crops on a global scale (Welch and Graham, 1996). Many types of soils with various characteristics such as high and low pH (pH between 4.5–7.5 are optimal for most of the plant species), high and low organic matter content (depending on the quality of organic matter), calcareous, sodic, sandy, wetland or poorly-drained, limed acid soils, etc. are reported as Zn deficient (Marschner and Marschner, 2012). These parameters encompass approximately half of the cereal producing soils worldwide (Alloway, 2004). Adequate reserves of mineral nutrients in seeds are needed to maintain seedling growth until the root system develops and takes over nutrient supply. Therefore, a good seed-mineral supply is beneficial, particularly for crops grown on nutrient deficient soils (Marschner and Marschner, 2012; Welch and Graham, 2004). Mineral Zn within seeds improves their viability, seedling vigor, and plant health leading to higher yields, particularly on Zn-deficient soils (Cakmak, 2008; Rengel, 2002; Rengel and Graham, 1995; Yilmaz et al., 1998). It also plays an important role in maintaining the structural stability of cell membranes against various stress factors (Marschner and Marschner, 2012). Furthermore, increasing the Zn content of cereal seeds is an efficient strategy for improving human nutrition and Zn intake. It is proposed that under Zn deficient soil conditions, high seed Zn content can protect against cell damage and decrease the leakage of various organic compounds to alleviate pathogen attraction that may cause pathogenic infection (Cakmak, 2000;

Abbreviations: DTZ, Diphenyle Thio-Carbazone; ICP-MS, Inductivity Coupled Plasma Mass Spectroscopy; LA-ICP-MS, Laser Ablation Inductivity Coupled Plasma Mass Spectroscopy

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Cakmak and Marschner, 1988; Welch et al., 1982). Therefore, sowing seeds with high Zn content can improve seedling health during early development and also avoid spatial and temporal variability in Zn availability under Zn deficient conditions (Rengel and Graham, 1995). The long-lasting benefits of high seed Zn content on plant growth are considerable as they increase grain yield (Imran et al., 2013; Rengel and Graham, 1995).

Zn bio-fortification of seeds is known to improve cereal nutrient levels, but evaluation of these seeds for agronomic traits, such as germination rate and early seedling development under various stress conditions has been less targeted. Therefore, for improving agronomic traits, seed treatment (seed priming and/or coating) with Zn has been shown as a useful tool for improving germination, early seedling development under various stress conditions, and final grain yields (Ajouri et al., 2004; Bradáčová et al., 2016; Harris et al., 2007; Imran et al., 2013, 2015; Prom-u-thai et al., 2012; Slaton et al., 2001). "Nutrient seed priming" is a technique in which seeds are soaked in a nutrient solution instead of pure water to enhance seed nutrient content along with the priming effect to improve germination and establishment. Seed priming with Zn increases seed Zn content, as has been shown for maize (Imran et al., 2015), barley (Ajouri et al., 2004), and rice (Prom-u-thai et al., 2012). It has been also shown in maize (Imran et al., 2015) and rice (Prom-u-thai et al., 2012) that primed Zn is translocated to growing shoots during germination and early seedling development. Furthermore, Harris et al. (2007) and Imran et al. (2013, 2015) has shown a significant increase in maize grain yield via Zn seed priming under low Zn available soils and low temperature climatic conditions, respectively.

The distribution of trace elements in seed grains is highly heterogeneous (Kyriacou et al., 2014; Lombi et al., 2011; Ozturk et al., 2006). The accumulation, distribution and localization of Zn and other trace elements in cereal grains, particularly in wheat is reported by numerous studies (Cakmak et al., 2010a; Ozturk et al., 2006; Persson et al., 2016, 2009; Wu et al., 2013). In wheat seeds, most of the Zn and other nutrient elements are accumulated in the embryo (Cakmak et al., 2010a; Wu et al., 2013). In maize seeds most of the mineral nutrients are located in the aleurone layer, but detailed information regarding Zn distribution in the maize seed is lacking.

Different state-of-the art analytical methods and techniques such as DTZ staining (Ozturk et al., 2006), and X-ray fluorescence and absorption techniques (Lombi et al., 2011; Neal et al., 2013) have been used to examine the localization and distribution of Zn and other elements in bio-fortified cereal grains. Recently, Ajiboye et al. (2015) have employed X-ray fluorescence microscopy (XRF) to show the Zn enrichment of wheat endosperm. According to Becker et al. (2008), despite a slightly lower resolution, state-of the-art laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) offers a higher sensitivity when compared to X-ray based techniques. In wheat grain, LA-ICP-MS has been used to explain the transportation and localization of Zn into the endosperm through crease phloem (Cakmak et al., 2010b). Most recently, Persson et al. (2016) have used LA-ICP-MS for the bio-imaging of Zn and its potential ligands in wheat grain from plants grown with increased Zn and N supplies.

Despite the above-mentioned research, it is not yet understood how primed Zn accumulates and is distributed in different seed tissues. The objectives of the present study were to determine the localization and distribution of Zn in various maize seed tissues in response to water and Zn priming treatments. This was observed through ICP-MS, DTZ staining, and LA-ICP-MS methods. Our hypothesis assumed that Zn is uniformly accumulated in maize seed during the imbibition process in a Zn solution.

2. Materials and methods

2.1. Seed priming

Seeds of *Zea mays* L. cv SunStar (provided by NPZ (Norddeutsche Pflanzenzucht) Hans-Georg Lembke KG, Germany) were used for Zn priming treatments. The seeds underwent a pre-determined 24-h priming by soaking in deionized water and 4 mM Zn solutions (Imran et al., 2013), respectively. Unprimed seeds were taken as a control treatment. The water absorbing capacity of maize seeds was 0.08 mL seed^{-1}. For water priming, 60 seeds were soaked in 200 mL of deionized water and for Zn priming, a 4 mM Zn solution of $ZnSO_4{\cdot}7H_2O$, was used. Thereafter, seeds were removed and rinsed with running distilled water for 1 min to remove adhering chemicals. The same priming method was used for all analytical techniques to determine Zn concentration and distribution in the seeds. Unprimed seeds were used as controls.

2.2. Zn measurement in whole maize seed, endosperm/embryo, and testa

For whole grain Zn analysis, unprimed, water, and Zn primed seeds were dried at 60 °C until a constant weight was achieved. To calculate average seed dry biomass, 20 maize seeds in 4 replicates were also dried at 60 °C to a constant weight. To analyze testa, and the remainder of the seed (endosperm/embryo), seed testa was carefully removed from water and Zn primed seeds immediately following 24-h priming treatments. The separated testa and endosperm/embryo were rinsed for 20 s with deionized water to remove surface adhering chemicals. Subsequently, the separated seed materials were dried at 60 °C till a constant weight was achieved. After being finely ground, 100 mg of each of whole grains, testa, and endosperm/embryo were digested with 10 mL of 69% HNO_3 (ROTIPU-RAN Supra for ICP, 69%) in a 1800 W microwave oven (MARS6 Xpress; CEM, Matthews, MC, USA) following the protocol of Jezek et al. (2015). Samples were diluted with 2% HNO_3 and total Zn was determined by inductively coupled plasma mass spectroscopy (ICP-MS; Agilent 7700, Agilent Technologies Inc., USA) as described by Jezek et al. (2015). Seed nutrient contents were calculated as follows:

Zn contents (μg seed^{-1}) = seed dry biomass x Zn concentration (μg g^{-1})

2.3. Staining of seed Zn

Staining of seed Zn was performed using DTZ (1,5-diphenyl thiocarbazone), according to Ozturk et al. (2006). For this purpose, unprimed and primed seeds were dissected into two halves with a sharp knife. Dissected seeds were then incubated with 500 mg L^{-1} DTZ at room temperature for 30 min. The stained seeds were rinsed with deionized water and analyzed qualitatively under a light microscope (DM2500 LED, Leica Microsystems, Wetzlar, Germany) with a mounted high-resolution digital camera (DFC495, Leica Microsystems, Wetzlar, Germany).

2.4. Localization of Zn in maize seeds using LA-ICP-MS

For LA-ICP-MS analyses, hot air dried (60 °C), unprimed and primed maize seeds were mounted in Specifix 40™ epoxy resin (Struers GmbH, Willich, Germany). For this purpose, cylindrical teflon moulds (Ø = 2.5 cm and 6 mm thickness) were initially filled to a 4 mm-high with epoxy resin. After partial hardening, seeds were horizontally placed on the semi-hardened resin, and then moulds were filled with resin liquid, so that half of the seed was merged out of the resin surface. After resin completely hardened, parts of the seeds emerged out of the resin surface were ground with SiC Foil, Grit 2000. (Struers GmbH,