Effect of Zinc Priming on Zinc Concentration of Germinating Rice Seed

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ABSTRACT

Zinc (Zn) in rice seed is important for its germination as well as to nutrition of rice eaters. The present study evaluated the effect of zinc priming, a pre-treatment of Zn of rice seed in 5 mM $ZnSO_4.7H_2O$ for 60 minutes, on its subsequent germination. The priming increased Zn concentration of whole rice seed by 3-4 fold, but not in the caryopsis (brown rice). The concentration of Zn in primed seed decreased after one day of germination and continued to decline from D2-D7, while it remained unchanged in unprimed seed for entire germination period. The density of Zn after priming with 5 mM could be monitored by the intensity of DTZ staining, when compared with those without Zn priming. As germination proceeded Zn concentration of the husk declined while that in the caryopsis increased ($r = 0.61$, $P < 0.05$). At the end of germination period, Zn concentration in the seed, coleoptiles and root of primed seed was 4.0, 1.6 and 2.0 fold higher than those from unprimed seed, respectively. Better growth of seedlings grown from seed primed with Zn was shown by dry weight of the coleoptiles and roots which was 0.80 and 1.02 mg seed^{-1} in primed seed and 0.60 and 0.83 mg seed^{-1} in unprimed seed, respectively. The results showed how priming rice seed with Zn effectively enhanced Zn concentration in rice seed as well as and improved Zn nutrition of coleoptiles and roots, suggesting a useful method to improve germination and seedling vigor, especially in rice seed that is low in Zn.

Key words: Rice, Seed germination, Zn concentration, Zn priming

INTRODUCTION

Zinc plays some very important physiological roles during seed germination and seedling development (Cakmak, 2008). It is essential in protein synthesis and gene expression (Broadley et al., 2007; Cakmak, 2000). Almost 10% of the proteins in biological systems need Zn for their structural and functional integrity (Andreini et al., 2006). During the seed germination, production of reactive oxygen species (ROS) is a well-documented phenomenon (Bailly et al., 2002; Cakmak et al., 1993; Qin and Liu, 2010) and Zn plays a central role in detoxification of ROS in plant cells (Broadley et al., 2007; Cakmak, 2000). On the other hand, rice seed has been reported to have very low Zn content compared with other cereals such as wheat and barley, which can result in adverse effects on seedling growth and development and grain yield as shown previously by Slaton et al. (2001). Previous studies reported that increasing seed Zn content prior to sowing in rice (Slaton et al., 2001), wheat (Rengel and Graham, 1995) and barley (Ajouri et al., 2004) significantly improved seed germination and seedling growth, especially when seeds are sown under Zn deficient soil. In addition to an improvement of seed germination and seedling growth, high seed Zn in rice also enhances the human nutrition and health which is estimated to affect about one-third of the world population, ranging from 4-73% of the countries' population, especially in developing countries where rice with low Zn concentration is predominantly consumed as the major staple food (Black et al., 2008; Hotz and Brown, 2004). Therefore, the concentration of Zn in rice seed requires further information for the improvement of both plant and human nutrition.

Currently, it has not yet been reported how priming Zn in rice seed effects on seed Zn concentration as well as seed germination. The present study set out to evaluate the effect of zinc priming, a pre-treatment of Zn of rice seed in 5 mM $ZnSO_4.7H_2O$ for 60 minutes, on its subsequent germination in comparison with un-primed seed Zn.

MATERIALS AND METHODS

Seed priming

Rice seed of cultivar TDK 7 was obtained from Laos PDR. Seed samples, each consisting of ten grams of seed (approximately 1000 seeds), were surface sterilized as follows. Each seed sample was washed 3 times with DDI water before soaking with 0.1% NaClO for 1 minute and transferred to 100 mL of 70% EtOH for 5 minutes. Then the seed was rinsed again 3 times with DDI water. Sterilized seed was soaked in 100 mL of $CaSO_4$ for 30 minutes before thoroughly rinsed again with DDI water. For the Zn priming procedure, seed samples were soaked in 100 mL of solution containing of 0 (Zn0) and 5 (Zn5) mM Zn in form of $ZnSO_4.7H_2O$ for 60 minutes. Primed seed was rinsed with DDI water and dried with tissue papers for their germination and analysis of Zn by inductively coupled plasma optical emission spectrometry (ICP-OES) (Vista-Pro Axial; Varian Pty Ltd, Mulgrave, Australia). For the ICP-OES analysis of Zn, exactly 10 seeds of paddy rice, brown rice and husk samples were first oven dried at 45°C and then subjected to acid-digestion in a closed-vessel microwave system (MarsExpress; CEM Corp., Matthews, NC, USA).

Seed germination

One hundred primed seeds of each Zn concentration and unprimed seeds were germinated between moist germination papers in plastic trays. Plastic trays were kept in a dark box. The germination period began when imbibition started (D0) and continued for the next 7 days (D7). The moisture inside germination trays was maintained by spraying DDI water daily. Germination rate, root and coleoptile length and dry weight were recorded daily over 8 days. The tests were conducted in four independent replications.

Diphenyl thiocarbazone (DTZ) staining

Ten seeds of paddy rice primed with 5 mM Zn kg^{-1} paddy rice of cultivar TDK 7 were submerged in freshly prepared DTZ solution by dissolving 1, 5 diphenyl thiocarbazone (Merck) 500 mg L^{-1} in methanol (AR grade) for 30 min as described previously (Ozturk et al., 2006) in comparison with unprimed Zn seeds (control). Samples were then rinsed thoroughly in DDI water and blotted to dry using tissue paper. The intensity of staining (red color) representing the relative density of Zn in the grains was assessed under an optical microscope (Nikon SMZ1500, Japan).

Data analysis

Analysis of variance was carried out to detect the difference among Zn treatments by using Statistic 8 (analytical software SXW). The least significant different (LSD) at $P< 0.05$ was applied to test for the significance difference among Zn treatments. The correlation analysis was used to test of pair-wise relationship.

RESULTS

Priming Zn in rice seed at 5 mM Zn increased Zn concentration in all seed tissues, excepted in brown rice compared with unprimed seed (Figure 1). Paddy rice of primed seed had about 5.5 fold higher Zn concentration than unprimed Zn seed, while it was about 32 fold in the husk and unchanged in brown rice.

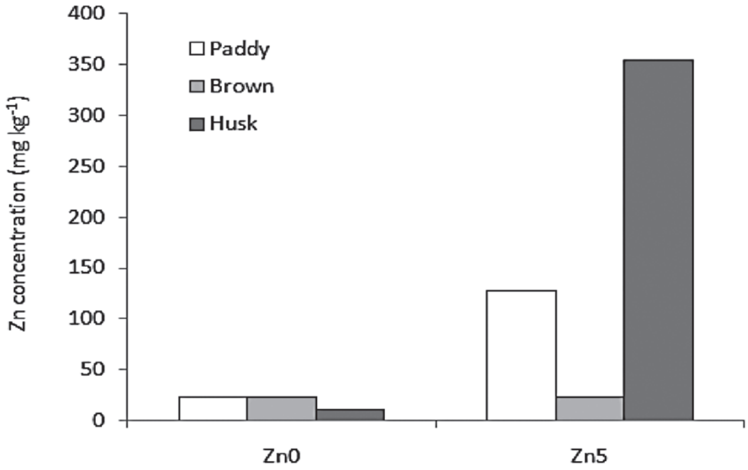

Figure 1. Zn concentration in different seed tissues of unprimed (Zn0) and Zn primed seed at 5 mM Zn (Zn5). Data represents the means of 4 independent replications. Different letters indicated the differences between each treatments at $P < 0.05$.

The concentration of Zn in primed Zn seed decreased after one day of germination and slightly declined from D1-D7, while it remained unchanged in unprimed Zn seed for the entire germination period (Figure 2).

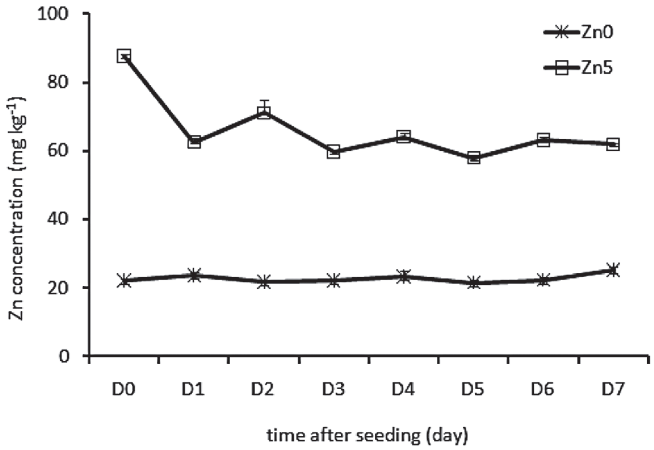

Figure 2. Zn concentration in paddy rice of cultivar TDK with 5 mM Zn priming (Zn5) and without Zn priming (Zn0) during 7 days after germination. Vertical bars represent standard errors of the means (±SE) of 4 independent replications.

On the other hand, Zn concentration of the husk declined with increasing Zn in the caryopsis (r = 0.61, $P < 0.05$) (Figure 3). Absorption and penetration of the primed Zn into the inner layer of the seed during germination and the growing embryo can be monitored by DTZ staining (Figure 4). The more intense red colour indicates higher Zn concentration on the rice husk after priming and the penetration of primed Zn on the germinating seed and embryo.

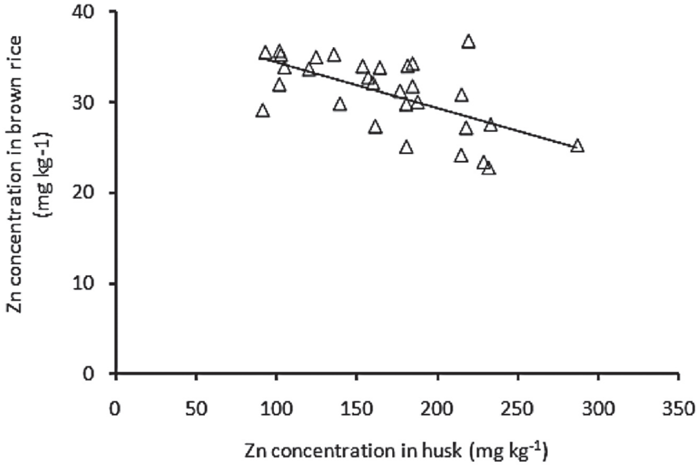

Figure 3. The relationship of Zn concentration between the husk and brown rice (B) of primed Zn seed (5 mM Zn) of cultivar TDK 7 during 7 days of the germination (n= 30).

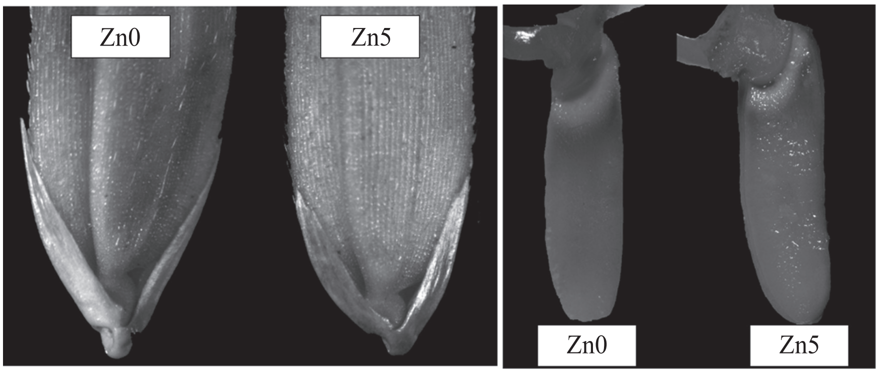

Figure 4. Stereo-micrographs of paddy rice without (Zn0) and with (Zn5) Zn priming of seeds stained with DTZ at the beginning of the germination (left) and 6 days after germination (right). The more intense red color indicates higher level of Zn in the seeds.

The concentration of Zn was different between unprimed and primed Zn seed among germinating tissues after 7 days of the germination ($P < 0.05$) (Figure 5). Zinc concentration in all tissues of primed Zn seed was 1.5-3.0 fold higher than in unprimed seed, depending on the tissue. In unprimed seed, Zn concentration in the coleoptiles and roots were similar (76 mg kg^{-1}), which were 3 times higher than the seed, while in the Zn primed seed the concentration of Zn was 81 mg kg^{-1} in the seed and 117 mg kg^{-1} in the coleoptiles and 148 mg kg^{-1} in the roots.

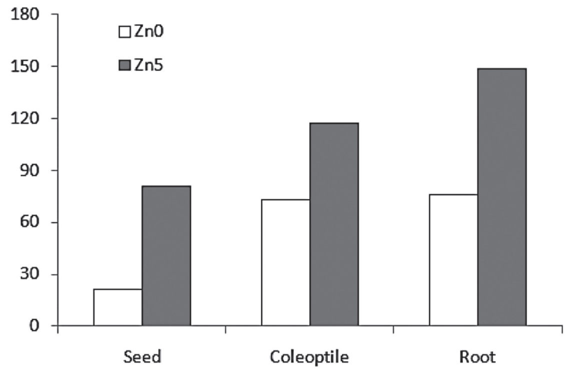

Figure 5. Zn concentration of germinated seed, coleoptiles and root of unprimed (Zn0) and primed (Zn5) at 7 days after germination period. The difference letters above bars indicated significant difference between Zn0 and Zn5 of different tissues (n=3).

Priming with Zn also increased dry weight of the rice seedlings (Figure 6). Dry weight of the roots was generally higher than coleoptile, but both were significantly increased by Zn priming ($P< 0.05$).

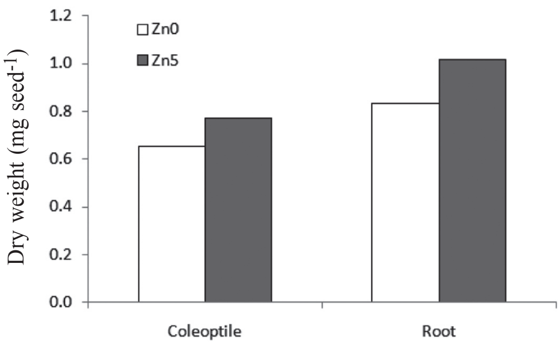

Figure 6. Dry weight of coleoptile and roots of the germinated unprimed (Zn0) and primed (Zn5) Zn seed at 7 days after germination. Difference letters indicated the significant different between primed and unprimed Zn seed of each grain tissue and Zn rate ($P< 0.05$) (n=3).

DISCUSSION

Priming Zn in rice seed effectively increased the concentration of Zn in rice seed, with important consequences on seed germination and seedling growth. Even though most of the primed Zn stayed on the rice husk right after priming, the evidence from the negative correlation between Zn concentration in the husk and caryopsis and the staining with DTZ clearly indicated that primed Zn penetrated into the internal grain layers during germination. It is possible that some primed Zn washed out from the outer grain layer resulted in declining of Zn concentration in rice seed, especially in the first day of the germination. Nevertheless, the concentration of Zn in primed seed was 3 folds higher than unprimed seed even after 7 days of the germination. That the primed Zn then became useful in physiological functions of the germinating seed was evident in the enhanced growth of the rice seedlings grown from the Zn primed seed.

Seed Zn has an important physiological role on seedling germination, seedling vigor and viability (Cakmak, 2008). Zinc is an integral component of enzyme structures which are involved in DNA replication and gene expression (Coleman, 1992). Thus, Zn deficiency in rice seed can have adverse effects on seedling growth and development. Previous studies reported that increasing seed Zn content prior to sowing in rice (Slaton et al., 2001), wheat (Rengel and Graham, 1995) and barley (Ajouri et al., 2004) significantly improved seed germination and seedling growth, especially when seeds are sown under Zn deficient soil. The present result in rice also indicated that higher seed Zn concentration from priming process enhanced dry weight of both roots and coleoptile tissues. More of the Zn in primed seed accumulated in the roots than the coleoptile. This can be explained by the theory that the tissues which are the most function such as in young leave for the mature plants and root for young seedling accumulate much higher nutrient concentration than the tissues which are not readily function, especially for the easy retranslocated nutrients (Marschner, 1995).

CONCLUSION

The present results indicate that priming Zn in rice seed effectively improved Zn concentration in rice seed, resulting in better growth and development of the seedling. The further studies should evaluate conditions in which Zn priming would help to increase grain yield and how the priming process might be incorporated into the routine preparation of rice seed prior to sowing.

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