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Research Article

The Efficiency of Zn Fertilization on Enhancing Grain Zn Mass Concentration in Aerobic Rice

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Abstract: Rice is a major source of energy and minerals for people whose diets are predominantly based on rice, to evaluate the potential role of Zn fertilization strategy (including Zn supply levels and ways) in increasing Zn concentration in rice grain need to be better understood and two so-called aerobic rice cultivars (Handao502 and Baxiludao) were used for this study. Field-condition experiment was carried out with eight Zn supply ways. Zn supply ways effect on shoot ZnMC was not significant neither for Baxiludao nor Handao502, while grain ZnMC was affect by Zn supply ways significantly, with foliar Zn supply grain ZnMC increased for both varieties and the grain ZnMC was increased to 21.37 mg/kg and to 24.21 mg/kg for Handao 502 and Baxiludao with only Foliar Zn application instead of Zn as soil applications. It could be concluded that in aerobic rice it is difficult to enhance grain ZnMC to reach the level human consumption demand by simply soil Zn supply or foliar Zn. Based on the relations among shoot Zn mass concentration, grain Zn mass concentration, Zn uptake and Zn supply, three approaches on enhancing grain Zn are suggested in this study.

Keywords: Aerobic rice, foliar Zn, *Oryza sativa L*, Zn mass concentration

INTRODUCTION

Zinc (Zn) deficiency in humans is widespread, affecting up to two billion people, over 30% of the world's population may suffer from zinc deficiency especially among resource-poor women and children who diet with agricultural products constituting the main source of essential minerals (Welch, 1993). In China, 76% of the zinc dietary is from plants and mostly is derived from consumption of cereals and legumes, however, the grain Zn concentrations are generally low and not adequate for human nutrition. Therefore, its increase is being considered as a sustainable, long-term solution to human Zn deficiency (Rengel *et al*., 1999). Earlier studies have shown that fertilizer strategy is an important complementary approach to increasing grain Zn mass concentration (mg Zn/kg dry matter). In wheat, it was found that combined application of Zn through soil and foliarly as $ZnSO_4$ both before and after anthesis was more effective and grain Zn concentrations increased up to 60 mg/kg (Cakmak, 2008). And more than threefold increase in grain concentrations of Zn in wheat following soil Zn application was also seen in Australia (Graham *et al*., 1992) and India (Shivay *et al*., 2008) under field conditions. However, in contrast to wheat, in rice the magnitude of the increases of grain Zn concentration increased by Zn fertilization in the field was not as big as in wheat (Yilmaz *et al*., 1997; Kalayci *et al*., 1999; Gao *et al*., 2006; Jiang *et al*., 2008a). And we found a poor translation in nutrient solution experiments of enhanced grain Zn concentration when we improved total plant uptake or total plant Zn mass concentration (Jiang *et al*., 2007; Jiang *et al*., 2008b; Stomph *et al*., 2009). Aerobic rice has been considered a promising rice water saving cultivation system and in large parts of China it can be expected that water limitations will lead to changes in the rice production technologies (Belder *et al*., 2005; Bouman *et al*., 2007). In fact in some of those areas soil pH is rather high potentially leading to low levels of Zn bioavailability (Gao *et al*., 2006). Under laboratory conditions poor re-allocation of foliar applied Zn was found in rice plant (Jiang *et al*., 2007; Wu *et al*., 2010), so we also want to check this under field conditions and in combination with soil Zn applications to see how the established correlation between total plant Zn and grain Zn was changed by the method of Zn application. Towards the

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end of the paper we would like to then indicate that the relations we found could be used to dissect grain Zn for breeding purposes into different routes and that characterising cultivars for each could help to seek for a combination of different reasons for an improved grain Zn.

MATERIALS AND METHODS

The experiment was carried out in Laiyang, Shandong province, China (36°58' N, 120°42' E) in 2008. The soil at the experimental site is a Meadow cinnamon soil with pH 6.8. DTPA-extractable Zn was 0.82 mg/kg soil. The experimental design was a split-plot, with three replications, main plots were eight Zn supply ways, i.e., 0 kg soil Zn (without foliar Zn(control), with foliar Zn), 100% soil Zn supply (11.32 kg/ha) as basal (with, without foliar Zn), 50% soil Zn supply (5.66 kg/ha) as basal +50% soil Zn supply (5.66 kg/ha) at about 7 days before flowering (with, without foliar Zn), 100% soil Zn supply (11.32 kg/ha) at 7 days before flowering (with, without foliar Zn). The total amount of Zn supply to the soil was 11.32 kg/ha. For the foliar application, it was applied twice (0.3% $ZnSO_4.7H_2O$solution containing 0.01% L-77, a leaf surface wetting agent): at flowering and 7 days after flowering. And subplots the same two rice accessions (Handao502 and Baxiludao) as used in the Zn supply levels experiment. Each subplot was 15 m^2, sown to 10 rows at an inter-row distance of 0.25 m and hole-distance of 0.10 m (two seeds per hole).

Composite fertilizer (N-P_2O_5-K_2O: 12-18-10) at the rate of 50 kg P/ha and 75 kg N/ha and Zn fertilizer (only in the +Zn plots) were incorporated before planting and 50 kg N ha^{-1} was top-dressed as ammonium nitrate at maximum tillering. Weeds were controlled by a pre-emergence herbicide and hand weeding. Plants were grown under rainfed conditions, with supplemental irrigation from a deep well, directly following sowing and at flowering, applied through flexible hoses connected to a subsurface pipe system.

Measurement items and methods: Plants were harvested at physiological maturity. Two 1-m segments of row were sampled per plot. The harvested plants were partitioned into stems including leave sheaths, leaf blades rachis and glumes combined and the brown rice grain.

All plant material was dried at 75°C for 48 h. Dried plant samples were ground in a stainless steel mill and passed through a 0.25-mm sieve before analysis. Sub-samples were digested in a bi-acid mixture (HNO_3: $HClO_4$ = 4:1). Zn was determined by atomic absorption spectroscopy (SPECTRAA-55; Varian Australia, Mulgrave, Australia).

The data from the solution culture experiment reported by Jiang *et al*. (2008b) together with the data of this Experiment 1 will be used for summarize the relationships among shoot Zn mass concentration, grain Zn mass concentration, Zn uptake and Zn supply under field and solution condition.

Data analysis: Regression analysis and Analysis of Variance (ANOVA) were performed with SAS (SAS Institute, 1989).

RESULTS AND ANALYSIS

Treatment effects on shoot dry matter and grain yield: No effect was observed ($p>0.05$) of either the Zn treatment or the cultivar on shoot or grain dry weight (Table 1). However, Among all Zn supply treatments, Handao 502 reached the highest shoot dry weight and grain weight with the treatment of 50% soil Zn supply as basal and 50% soil Zn supply at about 7 days before flowering with foliar Zn; Baxiludao reached the highest the highest shoot dry weight and grain weight with the treatment of 50% soil Zn supply as basal and 50% soil Zn supply at about 7 days before flowering without foliar Zn.

Treatment effects on shoot Zn mass concentration: Significant ($p<0.05$) differences were observed between the two cultivars with the higher shoot ZnMC in Baxiludao. The shoot ZnMC of Baxiludao was 23.2% higher than that of Handao502. However, neither the interaction nor the Zn treatment effect were significant $p>0.05$) (Fig. 1).

Table 1: Effect of Zn supply ways on dry matter production in shoot and grain for the two tested varieties, Handao502 and Baxiludao

	Shoot dry matter (kg/ha)		Grain yield (kg/ha)	
Zn supply ways	Handao502	Baxiludao	Handao502	Baxiludao
0 kg Zn	9696.4	9936.4	4130.2	4497.2
100% Zn as basal	9624.9	8095.1	4130.7	3245.8
50% Zn as basal 50% Zn at 7 DBF	9911.7	12319.6	4204.7	5549.8
100% Zn at 7DBF	8769.2	8617.5	3697.6	3422.2
0 kg Zn+ foliar Zn	10281.4	8770.0	4051.8	3591.0
100% Zn as basal + foliar Zn	9210.2	8419.7	3871.3	3048.7
50% Zn as basal + 50% Zn at 7 DBF+ foliar Zn	12567.5	8871.0	5447.2	3457.4
100% Zn at 7DBF+foliar Zn	9209.6	8892.0	3660.6	3409.7
Significance				
Variety	P = 0.29		P = 0.30	
Zn	P = 0.39		P = 0.39	
Variety*Zn	P = 0.46		P = 0.30	

Table 2: Effect of Zn supply ways on Zn mass concentration in individual organ

	Zn mass concentration (mg/kg)							
	Stem		Leave		Panicle structure		Grain	
Zn supply ways	Handao502	Baxiludao	Handao502	Baxiludao	Handao502	Baxiludao	Handao502	Baxiludao
0 kg Zn	41.13	50.40	21.97	21.12	25.88	25.60	16.60	18.44
100% Zn as basal	41.94	64.54	23.02	25.56	23.12	28.34	16.83	18.03
50% Zn as basal 50% Zn at 7 DBF	43.17	50.31	22.90	22.34	28.56	25.17	18.38	16.30
100% Zn at 7DBF	36.72	57.66	23.42	23.27	21.68	29.55	16.13	20.17
Average							16.99	18.24
0 kg Zn+foliar Zn							21.37	24.21
100% Zn as basal +foliar Zn							18.73	24.82
50% Zn as basal +50% Zn at 7 DBF +foliar Zn							18.55	22.95
100% Zn at 7DBF +foliar Zn							17.70	23.06
Average							19.09	23.76
Significance								
Variety	P = 0.0018		P = 0.7656		P = 0.1844		P = 0.0010	
Zn	P = 0.5569		P = 0.1480		P = 0.9474		P = 0.0002	
Variety*Zn	P = 0.4298		P = 0.4560		P = 0.1195		P = 0.1346	

DBF: Days Before Flowering; We do not have the chemical Zn analyses for the leaves, stems and panicle structure of the foliar treated plants

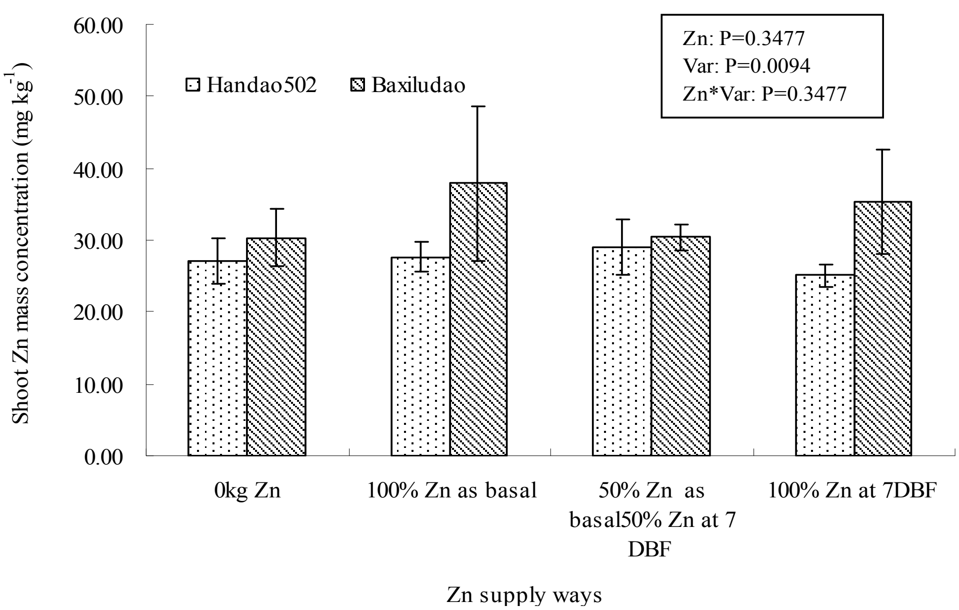

Fig. 1: The effect of Zn supply ways on shoot Zn mass concentration (Experiment 2) Error bars indicate±SD (n = 3) for individual data points

Treatment effects on Zn mass concentration of individual organ: Grain ZnMC was affect by Zn supply ways significantly, with foliar Zn supply grain ZnMC increased for both varieties (Table 2). Compared with control, the grain ZnMC was increased by 28.7 and 31.3% for Handao 502 and Baxiludao with Foliar Zn application without Zn as soil applications. Compared with soil Zn supply without foliar Zn, the average grain ZnMC increased by 12.38, 30.30% for Handao 502 and Baxiludao, respectively when foliar Zn was supplied. The Zn concentrations in stem were 2-3.5 times higher than that in grain. While the ZnMC was not significantly affected by Zn supply ways for either stem, leave, or panicle structure.

Treatment effects on Zn harvest index: The treatment effect on Zn harvest index was not significant at p = 0.05 neither for Baxiludao nor Handao502 and the difference between varieties in Zn harvest index was not significant (Fig. 2), the average Zn harvest index was 0.20 for Handao 502 and 0.18 for Baxiludao.

The relationships among shoot Zn mass concentration, grain Zn mass concentration, Zn uptake and Zn supply: The below described relations among shoot Zn mass concentration, grain Zn mass concentration, Zn uptake and Zn supply under field and solution culture condition in Fig. 3. It indicated that plants took up more Zn when Zn supply was increased

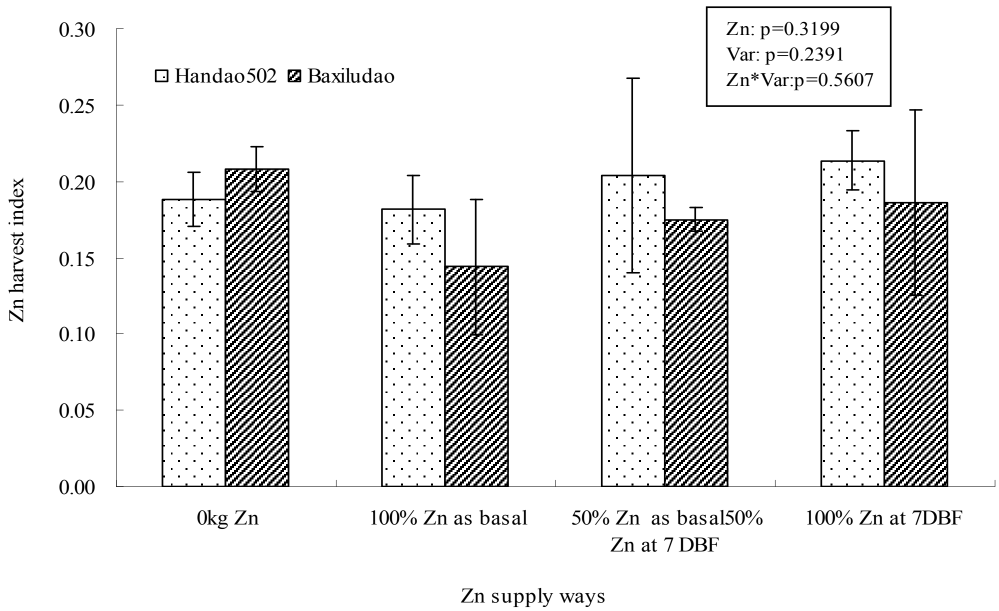

Fig. 2: The effect of Zn supply ways on Zn harvest index (Experiment 2) Error bars indicate±SD (n = 3) for individual data points

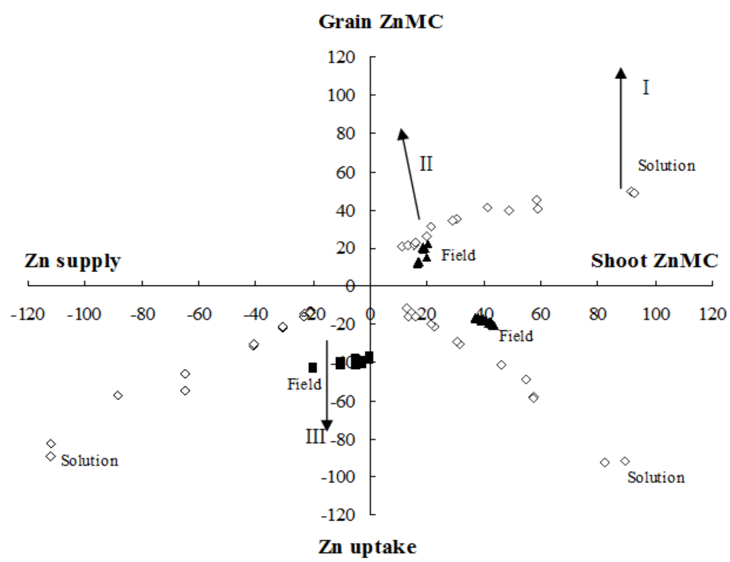

Fig. 3: The relationship between shoot Zn mass concentration (ZnMC) and grain Zn mass concentration, between Zn uptake and shoot Zn mass concentration, between Zn supply and Zn uptake under field and solution culture condition; Data from field grain-Zn mass concentration screening experiment with 14 cultivars (Jiang *et al*., 2008a), and from the solution experiment by seven Zn supply levels with two cultivars (Baxiludao and Handao502) (Jiang *et al*., 2008b); In the field: the unit for soil Zn supply is kgZn/ha, for Zn uptake is kgZn/5/ha, and for grain and shoot ZnMC is mg/kg; In solution: the unit for Zn supply is μg Zn/10/plant, for Zn uptake is μg/10/plant, for grain ZnMC is mg/kg, and shoot ZnMC is mg/2/kg

and shoot ZnMC increased proportionally with Zn uptake. However, the relationship between shoot Zn mass concentration and grain Zn mass concentration is logarithmic. At higher Zn uptake levels, the ZnMC in hulled grains increased least. Under field condition, although with Zn supply, the available Zn might be still low, which result in low plant Zn uptake and shoot Zn mass concentration, so there is some scope for increasing Zn uptake in the field.

DISCUSSION AND CONCLUSION

In this study, we found under field condition, although shoot Zn mass concentration (ZnMC) could be increased by both Zn supply levels and Zn supply ways, the highest shoot ZnMC was only 20.53 mg/kg and 19.62 mg/kg for Handao502 and Baxiludao, respectively, which is consistent with the report by Jiang *et al*. (2008a) in field aerobic rice Zn efficient screening. Therefore, we could conclude that under field condition shoot Zn ZnMC was still low and less than 50 mg/kg even surplus Zn supply to the soil in different ways, which might be mainly due to low Zn availability in the field condition.

Zn mass concentrations in grain was significantly affected by Zn supply levels and Zn supply ways, however, by surplus soil Zn supply (20 kg Zn/ha), grain ZnMC was only increased to 22.06 mg/kg and to 15.41 mg/kg for Handao502 and Baxiludao. Meanwhile, Although Zn foliar Zn supply indeed increased grain ZnMC, compared with single soil Zn supply without foliar Zn, average grain ZnMC increased by 12.38, 30.30% for Handao 502 and Baxiludao, respectively when foliar Zn was supplied. However, even with foliar Zn supply, the average grain ZnMC was only 19.09 mg/kg and 23.76 mg/kg for Handao 502 and Baxiludao, respectively. Therefore, in rice, Zn unloading in phloem is different from wheat (Stomph *et al*., 2009) and phloem transport of Zn from leaves was not an important as xylem transport of Zn from root for grain filling in rice (Jiang *et al*., 2007; Wu *et al*., 2010).

In rice, therefore, fertility management alone will not sufficiently enhance grain Zn density and further efforts are needed. Looking at the graph (Fig. 3), three approaches are proposed here, the first option is to simply ‘breed for’ an enhanced level of “maximum concentration at high plant zinc” (lifting the line along the vertical axis, the arrow I) or by increasing the responsiveness of the grain Zn in relation to plant Zn levels (the arrow II). A third is that with the same soil Zn plants take up more and thus reach a higher plant Zn concentration at a lower soil Zn level (the arrow III). The first and second relate to enhancing the Zn harvest index or allocation efficiency, where changing the shape towards a steeper initial reaction would seem most effective. The third option is a matter of improving plant uptake, so potentially governed by a different set of genes, which might be attained through breeding.

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