Title

Malate exudation by aerobic rice (Oryza sativa L.) grown on a low Zn soil

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Introduction

Low zinc (Zn) availability and water availability are constraint to lowland rice (*Oryza sativa* L.) production in many areas of the world. Aerobic rice varieties are newly bred by crossing lowland with upland varieties and are grown as a dry field crop in irrigated but non-flooded and non-puddled soils. The introduction of aerobic rice on low Zn soils puts the problem of Zn deficiency in rice in a new perspective. Research on mechanisms of Zn acquisition by plants is needed to develop rice varieties with high tolerance to low Zn soils.

Rice roots can release low molecular weight organic anions (LMWOAs) into the rhizosphere. These root exudates have been implicated in many processes, such as nutrient mobilization, detoxification of heavy metals, and mineral weathering. Recently, lowland rice was found to respond similarly to P and Zn deficiency by increasing citrate exudation and it was suggested that the LMWOA exudation capacity of rice genotypes was related to their tolerance to Zn deficiency (Hoffland et al., 2006). There is, however, little direct evidence for the role of root exudates in mobilizing Zn from low soils.

In this study, we investigated the LMWOA exudation of three aerobic rice genotypes in relation to their Zn uptake efficiency from a low Zn soil. We tested the following hypotheses: i) roots of aerobic rice exude more LMWOAs as a response to Zn deficiency; ii) genotypic variation in Zn uptake is related to LMWOA exudation.

Materials and Methods

A rhizotron experiment was conducted in a growth chamber. The experimental design was a factorial design with two Zn levels (0 and 5 mg Zn kg^{-1} soil as $ZnSO_4$) and three aerobic rice genotypes (K150, 91B and Hongke) with three replicates. The three genotypes showed variation in Zn uptake in previous experiments in the order of Hongke > 91B > K150.

Rhizotrons with an inner volume of 900 cm^3 were filled with a clay soil. The soil was classified as Luvisol with pH_{H2O} 6.5, organic matter 1.7%, DTPA-extractable Zn 0.3 mg kg^{-1}. Soils were air-dried, sieved at 5 mm, homogenized and moistened at 20% (w/w, equivalent to field capacity). One side of the rhizotron consisted of a PVC sheet with 5×5 mm grid of holes (1.8 mm) for insertion of micro-suction cups. The opposite side was made of a transparent Plexiglass plate and was covered by a removable dark PVC sheet. The purpose was to observe root growth and locate the sampling points. During the experiment, the rhizotrons were positioned at an angle of 30º to force the roots to grow along the Plexiglas plate side.

Four seeds of one genotype were sown per rhizotron containing 1.3 kg dry soil. At sowing time, each rhizotron received a basal application of 150 mg kg-1 N as $Ca(NO_3)_2$, 44 mg P as KH_2PO_4 and 83 mg kg^{-1} K as KCl. The plants were thinned to two seedlings per rhizotron one week after emergence. The soil was watered daily with deionized water, maintaining water content at 20% (w/w).

The soil solution was sampled by micro-suction crops (Shen and Hoffland, 2007) at the 28th day after sowing, when symptoms of Zn deficiency for -Zn plants and a growth response to Zn application were visual. For each treatment, nine root tips (three tips per replicate) were selected to collect the rhizosphere soil solution. Another two points, 3 cm away from the root system, were selected as bulk soil. Low pressure was build up inside each micro-suction cup using a 5 mL syringe. The pressure was maintained for 2 hours. The volume of sampled soil solution was around 500 µL for each micro-suction cup.

LWMOAs in the soil solution, including tartrate, malate, lactate, acetate, maleate, citrate, succinate and fumarate, were analyzed by reversed phase HPLC in the ion suppression mode. Plant shoots were harvested and dried at 70 °C for 72 h and weighed. Zinc efficiency was calculated as the ratio of shoot dry weight at Zn deficiency over that at adequate Zn supply.

Results and Discussion:

Zinc application increased shoot dry weight of Genotypes K150 and 91 B, but didn't affect that of Hongke (Table 1). Plants in –Zn treatment showed typical Zn deficiency symptoms, indicating plants in –Zn treatment suffered from Zn deficiency. Zinc efficiency based on shoot dry weight under rhizotron conditions was 55, 69 and 91% for genotypes K150, 91B and Hongke, respectively. There was no interaction between Zn levels and genotypes.

Table 1 Shoot dry weight and Zn efficiency of aerobic rice genotypes.

Genotypes	Shoot dry weight (mg plant^{-1})		Zn efficiency
	-Zn	+Zn	(%)
K150	151 b	274 a	55
91B	217 b	316 a	69
Hongke	275 a	302 a	91
ANOVA			
Sources	DF	$Pr \geq F$	
Zn levels	1	< 0.0001	
Genotypes	2	< 0.0001	
Zn × Genotypes	2	0.15	

Means followed by the same letter are not significantly different for –Zn and +Zn treatments (Tukey, $P = 0.05$)

Malate concentration in rhizosphere was significantly increased by Zn deficiency for genotypes 91B and Hongke, but not for genotype K150 (Figure 1). In general, malate concentration in the rhizosphere of three genotypes at Zn deficiency averaged 0.46 mM, which was 64% greater that at adequate Zn supply, indicating that aerobic rice may respond to Zn deficiency with increased root exudation of malate. However, linear regression analysis showed no relationship between rhizosphere malate concentration and plant Zn uptake for the three genotypes ($R^2 = 0.19$, $P = 0.72$), suggesting that Zn mobilization cannot be explained by increased malate exudation and the variation in plant Zn uptake is most likely not be explained by their capacity to exude malate as a response to Zn deficiency.

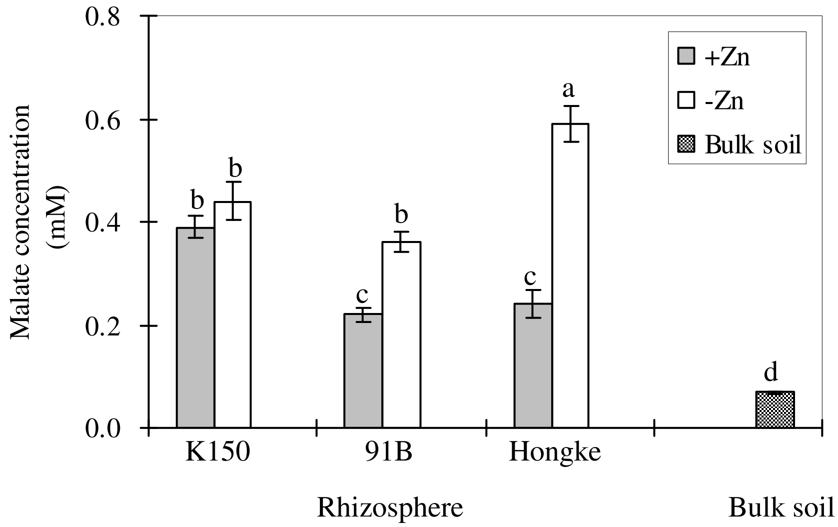

Figure 1 Malate concentration in the rhizosphere and bulk soil solution of three aerobic rice genotypes. Means followed by the same letter are not significantly different (Tukey, $P = 0.05$). The genotypes are presented in order of increasing Zn efficiency.

References:

Hoffland E, Wei CZ, and Wissuwa M. Citrate exudation by lowland rice (*Oryza sativa* L.) at zinc and phosphorus deficiency. Plant Soil 2006; 283:155-162.

Shen JB and Hoffland E. *In situ* sampling of small volumes of soil solution using modified micro-suction cups. Plant Soil 2007; 292:161-169.