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Organic anion exudation by lowland rice (*Oryza sativa* L.) at zinc and phosphorus deficiency

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Abstract

The objectives of this paper were to determine (1) if lowland rice (*Oryza sativa* L.) plants respond similarly to low zinc (Zn) and phosphorus (P) availability by increased root exudation of low-molecular weight organic anions (LMWOAs) and (2) if genotypic variation in tolerance to low soil supply of either Zn or P is related to LMWOA exudation rates. Exudation of LMWOAs can increase bioavailability of both Zn and P to the plant, through partly similar chemical mechanisms. We used seven lowland rice genotypes and showed in two experiments that genotypes that grow relatively well on a soil with low Zn availability also grow well on a sparingly soluble Ca-phosphate ($r = 0.80$, $P = 0.03$). We measured exudation rates of LMWOAs on nutrient solution and found that both Zn and P deficiency induced significant increases. Among the LMWOAs detected oxalate was quantitatively the most important, but citrate is considered more effective in mobilizing Zn. Citrate exudation rates correlated with tolerance to low soil levels of Zn ($P = 0.05$) and P ($P = 0.07$). In a low-Zn-field we found an increased biomass production at higher plant density, which is supportive for a concentration-dependent rhizosphere effect on Zn bioavailability such as LMWOA exudation. We, for the first time, showed that tolerance to low Zn availability is related to the capacity of a plant to exude LMWOAs and confirmed that exudation of LMWOAs must be regarded a multiple stress response.

Introduction

Zinc (Zn) deficiency is the most widespread micronutrient disorder in rice (Doberman and Fairhurst, 2000). It is common in flooded soils because of the reducing conditions that develop following submergence, with Zn becoming immobilised with ferrous iron compounds and other solid phases formed in reducing processes. In lowland rice Zn deficiency causes multiple symptoms that usually appear 2–4 weeks after transplanting of rice seedlings. Leaves develop brown spots and streaks that may fuse to cover older leaves entirely, plants remain stunted and in severe cases may die, while those that recover show a substantial delay in maturity and a reduction in yield.

Zinc deficiency is generally corrected by applying Zn sulphate to the soil. Zn application, however, is not always adequate to fully recover rice yields in genotypes with low tolerance (Van Breemen and Castro, 1980). Also, Zn fertilizers are unaffordable for many farmers. It has therefore been suggested to intensify breeding efforts to improve tolerance to low Zn availability (Neue et al., 1998).

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Tolerance to low Zn availability in plants is still poorly understood and many potential mechanisms have been proposed (Hacisalihoglu and Kochian, 2003). Tolerant cultivars may have lower Zn requirements, a relatively high translocation of Zn from roots to shoots, solubilise immobile forms of Zn in the rhizosphere, or possess a higher affinity for Zn, which would increase Zn uptake from soils with low Zn availability. An alternative attempt to explain tolerance to low soil Zn levels in rice focuses on the negative effect bicarbonate has on the growth of intolerant genotypes. Intolerant genotypes would show strongly reduced root growth at increased bicarbonate levels caused by submergence, which would inhibit new root initiation and thus Zn uptake (Hajiboland et al., 2003; Yang et al., 1993). Recently, this reduced root growth was attributed to excessive accumulation of organic acids in the roots of intolerant genotypes (Hajiboland et al., 2005). More tolerant genotypes would not show this accumulation as a response to bicarbonate due to higher transport and root exudation of organic acids.

Recently, involvement of a rhizosphere process in tolerance of aerobic rice to low Zn availability was suggested (Gao et al., 2005). Rhizosphere processes could significantly affect Zn bioavailability to rice (Kirk and Bajita, 1995). Hypothetically, reduction of the rhizosphere pH and root exudation of Zn chelators such as siderophores and low-molecular weight organic anions (LM WOAs) could increase Zn availability and uptake. There are several studies indicating a role of siderophores in tolerance to Zn deficiency in graminaceous plant species (Erenoglu et al., 2000; Hopkins et al., 1998; Rengel and Römheld, 2000; Rengel et al., 1998) but we are not aware of any study in rice. We are also unaware of studies on the role of LMWOAs in tolerance to Zn stress in any plant species. LMWOAs can increase soil Zn availability in two ways. First, plants exude LMWOAs by plant roots together with cations (generally protons) to maintain electrical neutrality (Jones, 1998; Raghothama, 2000). These protons reduce the rhizosphere pH, which increases Zn bioavailability, specifically in alkaline soils where Zn deficiency problems are most severe. Although the pH drop at the root surface in flooded soils will be small due to fast diffusion of acidity into the bulk soil, protons are assumed responsible for a major part of the Zn taken up by the plant (Kirk and Bajita, 1995). Second, LMWOAs are weak chelators of Zn and complexation of Zn may also increase Zn bioavailability. But they are strong chelators of Fe^{III}. This may play a role in lowland rice, because of the Fe plaque that is usually formed on the surface of roots. Zn can be adsorbed on this Fe plaque (Zhang et al., 1998). Chelation of Fe may release this Zn, although LMWOA can also be adsorbed on this Fe plaque, making them less effective as chelator.

Rice plants are known to exude LMWOAs (Aulakh et al., 2001). Similar to white lupin (Gardner et al., 1983), alfalfa (Lipton et al., 1987) and rape (Hoffland et al., 1989) citrate exudation by rice is increased as a response to phosphorus (P) deficiency, leading to increased P uptake (Kirk et al., 1999). The increase in P bioavailability due to exudation of citrate can be explained by mechanisms similar to the ones described above for Zn: sparingly soluble Ca-phosphates may dissolve due to a pH decrease (Gardner et al., 1983; Hoffland, 1992) or precipitation of Ca-citrate (Dinkelaker et al., 1989), and metal(hydr)oxide-sorbed phosphate can be mobilised by competitive binding of citrate (Geelhoed et al., 1998) or through chelation of metal ions (Kirk et al., 1999).

Zn deficiency often co-occurs with P deficiency, specifically on calcareous soils. Previously it was suggested that Zn deficiency tolerant rice genotypes show at least moderate tolerance to P deficiency and vice versa (Quijano-Guerta et al., 2002). This inspired us to investigate if this multiple deficiency tolerance can be explained mechanistically by exudation of citrate and other LMWOAs. The objectives of the present study are to (1) find out if a rhizosphere process may be involved in tolerance to low Zn availability in the field, to determine if (2) rice plants respond similarly to Zn and P deficiency by exudation of LMWOAs and (3) if genotypic variation in tolerance to low availability of either Zn or P is related to LMWOA exudation rates.

Materials and methods

Genotypes

Seven genotypes of rice (*Oryza sativa* L.) were selected for this study based on preliminary

experiments on Zn efficiency at the IRRI. Dular, a traditional variety from India, was chosen as the Zn deficiency intolerant check whereas M79, a modern breeding line of Chinese origin, had been classified as tolerant to low Zn availability. The remaining genotypes were selected from a mapping population that is intended to be used for QTL identification under Zn deficiency. This mapping population was derived from a cross of IR74 (modern variety, intolerant) with Jalmagna (traditional variety, tolerant). Lines of the mapping population were advanced to the F8 generation by single seed descent. A sub-sample of the mapping population (lines 474, 507 and 614) was used together with both parents.

Tolerance to low Zn availability

Field experiments were conducted on a highly Zn-deficient field located in Tiaong, Quezon province, Philippines. The field is characterised by low Zn availability (0.1 mg kg^{-1} DTPA-Zn), high pH (7.8; 1:1 w/v H_2O), high organic matter (2.8%) and a low redox potential caused by permanent flooding. Experiments were conducted in the dry season (January–April) of 2003. Twenty-day-old seedlings of the seven genotypes were transplanted in rows of 20 individual plants with spacing of 20 cm within and between rows. In addition, we transplanted bunches of five and ten seedlings at the head of each row to evaluate the effect of increasing plant (root) density on tolerance to low soil levels of Zn. A fertilised plot (15 kg Zn ha^{-1} $year^{-1}$) adjacent to the unfertilised plot was used as a +Zn treatment. The experimental design was a randomised complete block with four replications. Five weeks after transplanting three individual plants were sampled per row, roots and shoots were separated, oven dried at 70 °C for 4 days and dry weight was recorded. Plant samples were ground to a fine powder and 0.5 g tissue was extracted in 10 ml of 1 N HCl for 24 h. The extract was filtered and the Zn concentration in the filtrate was analysed by AAS. Zn efficiency was calculated for individual plants by dividing shoot dry weight at −Zn by shoot dry weight at +Zn. The remaining plants, including five- and ten-plant hills, were harvested 12 weeks after transplanting and measurements were repeated as described above.

Tolerance to low P availability

A pot experiment with quartz sand as substrate was done to determine the P efficiency of the seven rice genotypes. P efficiency is used as a quantitative parameter to express tolerance to low P availability and is defined as the capacity to grow well on a sparingly soluble P source. Treatments were rock P (RP) and KH_2PO_4 (+P). For each pot 540 ml nutrient solution was mixed with 3 kg quartz sand. The complete nutrient solution contained (in mmol L^{-1}) 4.0 KH_2PO_4, 5.0 KNO_3, 5.0 $Ca(NO_3)_2$, 2.0 $MgSO_4$ and (in mg L^{-1}) 0.5 B as H_3BO_3, 0.5 Mn as $MnSO_4$, 0.05 Zn as $ZnSO_4$, 0.02 Cu as $CuSO_4$, 0.01 Mo as $(NH_4)_6Mo_7O_{24}$ and 4.6 Fe as Fe-EDTA. The pH was adjusted to 6.5. In the rock phosphate (RP) treatment KH_2PO_4 was replaced by 570 mg Mali rock phosphate (a sparingly soluble apatite, P% = 13.4%) which was mixed with 3 kg of sand per pot. Six rice seeds were sown in each pot. After emergence, three weaker seedlings were discarded. Water content was adjusted daily according to weight loss. Three replicates were used. Rice plants were harvested after 50 days and dried at 70 °C for 72 h. P efficiency was calculated by dividing shoot biomass at RP by shoot biomass at +P.

Plant cultivation on nutrient solution

Rice seeds were sown in moist quartz sand. Six days after emergence, seedlings were transplanted to 50-L containers filled with a continuously aerated nutrient solution. The complete nutrient solution (+P, +Zn) contained (in mmol L^{-1}): 1.5 KH_2PO_4, 2.5 KNO_3, 2.5 $Ca(NO_3)_2$, 1.0 $MgSO_4$, and trace elements as given above. The pH was adjusted to and maintained at 6.5 by daily titration. In the −Zn and −P treatment $ZnSO_4$ or KH_2PO_4 were omitted, respectively. Thirty plants were grown per 50-L container until significant visual symptoms of Zn or P deficiency showed, which was around 25 days after transplanting.

Collection of root exudates

Roots of six plants grown on nutrient solution were rinsed with demineralised water and put individually ($n = 6$) in a test tube containing 20 mL demineralised water for 30 min. After

30 min the plants were removed, and the solution was filter-sterilised (0.2 μm). The first 10 mL that passed the filter were discarded to avoid effects of filtering on the citrate concentration. The sterile samples were stored immediately at −18 °C until analysis. Plant roots and shoots were oven dried at 70 °C for 72 h, and weighed. This experiment is referred to as Experiment 1. In a replicate experiment (Experiment 2) root exudates were collected on 20 mL plant^{-1} freshly prepared nutrient solution (composition: see above) during 2 h. To compare the exudation rates in these two experiments data were normalised such that the maximum exudation rate found in each experiment was set at 1.0. If not mentioned, results are from Experiment 1.

Citrate analysis

Citrate concentration in root exudates was measured spectrophotometrically using an enzymatic method. In a cuvette containing the sample and a glycylglycine buffer (pH 7.8) citrate lyase was added to convert citrate to oxaloacetate. Oxaloacetate and its decarboxylation product pyruvate were reduced by NADH to malate and lactate by addition of malate dehydrogenase and lactate dehydrogenase, respectively. The amount of NADH oxidised in these reactions is stoichiometric with the amount of citrate in the sample. The decrease in NADH in the cuvette was determined by means of its absorption at 340 nm. The detection limit for this assay is 0.5 μM. All chemicals were from Roche Diagnostics GmbH, Mannheim, Germany. The analysis was done on each of the six replicates.

LMWOAs analysis

A larger spectrum of LMWOAs, including oxalate, malonate, fumarate, tartrate, malate, succinate and maleate was analysed with a capillary ion analyser (Waters, Milford, MA, USA), according to Westergaard et al. (1998). The detection limit for each individual LMWOA is around 1 μM. Citrate cannot be analysed under these conditions because it is a stronger acid than the other acids. The analysis was done on three replicates randomly chosen from the six replicates of root exudates.

Data analysis

Statistical analysis of data was performed with SPSS analytical software (SPSS Inc., Chicago, IL, USA; version 12). Analysis of variance (General Linear Model) was used to find main effects and Tukey HSD to test differences among treatments. Data on exudation rates were log transformed to meet the requirement of homogeneity of variance.

Results

Field data showed a large variation in biomass production among the genotypes tested on a soil with low Zn availability (Figure 1). At low plant density Dular and IR74 produced less dry matter than Jalmagna, M79 and 507. Genotypes 614 and 474 were intermediate. Plant density had a large impact on biomass production. An increase from 1 to 10 plants per hill resulted in an up to five-fold increase in shoot biomass. This increase co-occurred with a reduction in Zn deficiency symptoms (data not shown), indicating that higher biomass production is caused by higher Zn uptake.

Genotypic variation in field biomass production (Figure 1) could be explained by variation in tolerance to low Zn availability (Table 1). Genotypes known to be intolerant (Dular and IR74) only produced about 10% shoot biomass under Zn-deficient conditions compared to sufficient Zn supply, but the tolerant genotypes 507, M79 and Jalmagna had a Zn efficiency of 40%. That the reduction in biomass was due to Zn deficiency was evident from the comparison of Zn concentrations in shoot tissue. The average concentration in the +Zn control was 44.1 μg g^{-1} but this dropped to 10.8 μg g^{-1} in the −Zn treatment. Total Zn uptake was reduced by up to 95% in intolerant genotypes (data not shown).

The variation among the genotypes in capacity to use rock P as P source was similar. P efficiency ranged from 37 to 67%. Generally, genotypes with a low Zn efficiency also showed a low P efficiency: Zn and P efficiency were positively correlated ($r = 0.80$, $P = 0.03$).

Main effects of nutrient supply and genotype on citrate exudation rate were highly significant (Table 2). Citrate exudation increased at P or Zn

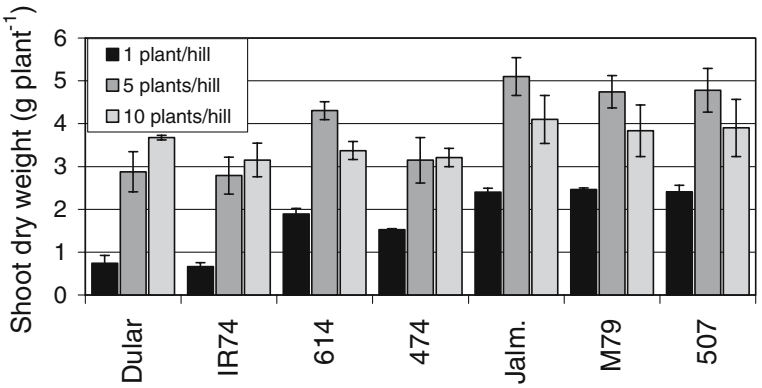

Figure 1. Biomass production and effect of plant density of rice genotypes in a strongly Zn-deficient field.

Table 1. Tolerance to low Zn and P availability, expressed as Zn and P efficiency, of seven lowland rice genotypes

Genotype	Zn efficiency (%)	P efficiency (%)
Dular	9	37
IR74	13	37
614	34	49
474	36	43
Jalmagna	40	58
M79	40	67
507	42	52

Efficiencies are calculated for individual plants as (shoot dw at low nutrient supply)/(shoot dw at high nutrient supply) $\times 100$.

Table 3. Effect of nutrient supply on root citrate exudation averaged for seven genotypes

Nutrient supply	Citrate exudation rate	
	(nmol g^{-1} plant dw h^{-1})	(nmol g^{-1} root dw h^{-1})
Complete (+P; +Zn)	0.22 A	0.9 A
−Zn	0.33 B	1.13 B
−P	0.33 B	1.72 C

Means with same letter are not significantly different (Tukey, $P < 0.05$).

deficiency. The highest exudation rates expressed per g root dw were found at P deficiency (Table 3). The average increase in citrate exudation was 82% at low P and 21% at low Zn. The interaction term nutrient*genotype was significant (Table 2). This indicates a stronger response for genotypes more tolerant to low nutrient availability.

Genotypes more tolerant to low soil Zn levels indeed tended to exude higher amounts of citrate ($P = 0.05$; Figure 2). This also holds for genotypes that are more P efficient ($P = 0.08$). Considering results of all genotypes from two experiments, the rate of citrate exudation at P deficiency was positively correlated with the rate of citrate exudation at Zn deficiency (Figure 3; $r = 0.87$, $P = 0.008$). Table 4 shows that, generally, also the response to Zn deficiency is generally stronger in genotypes more tolerant to low Zn availability.

In addition to citrate, other LMWOAs were analysed as well. Similar to citrate, exudation rates increased at nutrient deficiency (Table 4) though values on individual LMWOAs were

Table 2. ANOVA for citrate exudation rates, after log transformation

Source	D.F.	nmol g^{-1} plant dw s^{-1}		nmol g^{-1} root dw s^{-1}	
		F	*P*	*F*	*P*
Nutrient supply[1]	2	29.87	0.000	41.78	0.000
Genotype	6	15.33	0.000	4.43	0.000
Nutrient supply * Genotype	12	6.32	0.000	3.22	0.001

[1]Complete (+P, +Zn), −P or −Zn.

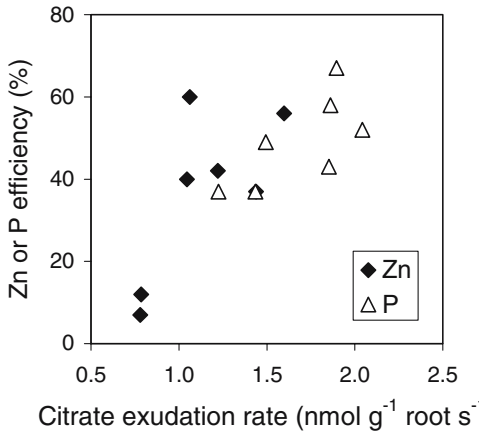

Figure 2. Relationship between citrate exudation on nutrition solution and Zn or P efficiency on a solid substrate for seven genotypes. For Zn: $r = 0.75$, $P = 0.05$. For P: $r = 0.70$; $P = 0.08$. Each marker represents one genotype.

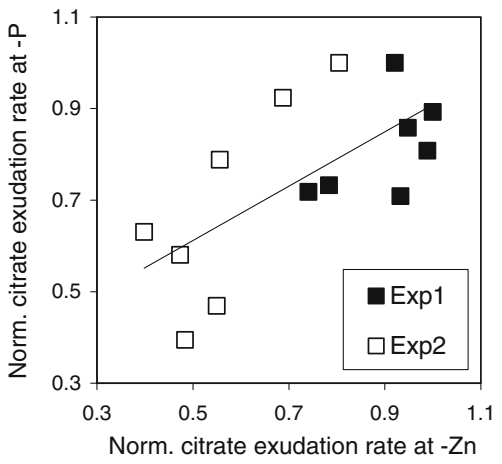

Figure 3. Relationship between citrate exudation rate at −Zn and −P. Data are from independent experiments and therefore normalised per experiment ($r = 0.87$; $P < 0.01$).

highly variable. Exudation rates of oxalate were generally higher than those of citrate (Table 3). There was no correlation between Zn or P efficiency of genotypes and their oxalate exudation response to Zn ($P = 0.3$) or P deficiency ($P = 0.9$), respectively. The same (absence of correlation) was true considering the sum of oxalate, citrate, malate, tartrate, malonate, fumarate, maleate and succinate ($P = 0.2$ for the correlation between Zn efficiency and exudation, and $P = 0.8$ for the correlation between P efficiency and correlation).

Discussion

We showed that lowland rice genotypes that grow relatively well on a soil with low Zn supply also grow well on a sparingly soluble P source (Table 1). This confirms earlier results with another set of genotypes (Quijano-Guerta et al., 2002) and is suggestive for common stress mechanisms. Citrate exudation rate significantly increased at Zn or P deficiency. Exudation rates per plant were mainly increased because roots were more actively exuding (Table 3), and not just because of higher root biomass. Generally, genotypes that show a high citrate exudation rate at Zn deficiency also show a high rate at P deficiency (Figure 3). So citrate exudation as a common response to nutrient deficiency may be part of a mechanistic explanation for the relationship between tolerance to low soil Zn and P supply.

We, for the first time, showed that plants respond to Zn deficiency with increased root exudation of LMWOAs. The pattern of the response is similar to the response to P deficiency (Tables 2–4). Citrate was the only LMWOA whose exudation rates related to Zn and P efficiency (Figure 2). Quantitatively, oxalate is the main LMWOA identified in the exudates. Citric acid, however, is a stronger acid than oxalic acid. At cytosolic pH (7) citrate is in the form of $cit^{-2.8}$, while the oxalate is in the form of $ox^{-2.0}$. This means that each citrate ion exuded will be countered by 0.8 charge equivalents of cations more than oxalate. If the counter ion is a proton, then citrate has a higher rhizosphere acidifying potential. In addition, at pH 7 citrate is a much stronger (7-fold) chelator of Zn^{2+}, and of Fe^{3+}. Careful modelling is needed to evaluate the impact of exudation of citrate and oxalate on Zn bioavailability to the root but it is likely that the increased exudation rates measured for citrate have a larger impact than those of oxalate.

The finding that biomass production is higher at higher plant density (Figure 1) is strongly supportive for a concentration-dependent rhizosphere effect on Zn uptake under field conditions. Higher root density may, apart from increased LMWOA soil concentrations, also increase the interception of solubilised Zn diffusing away from the rhizosphere. Increasing plant density from one to five plants per hill increased shoot dry matter per plant in a similar fashion

Table 4. Exudation rates (nmol g^{-1} plant dw s^{-1}) of LMWOAs

LMWOA	Nutrient supply	Genotype							Average[1]
		Dular	IR74	614	474	Jalm.	M79	507	
Citrate	Complete	0.2	0.2	0.2	0.2	0.3	0.2	0.1	0.2 ± 0.0
	−Zn	0.2	0.2	0.4	0.5	0.3	0.4	0.4	0.3 ± 0.1
	−P	0.3	0.3	0.4	0.5	0.3	0.2	0.3	0.3 ± 0.0
Oxalate	Complete	0[2]	0	0.8	0	0	0	0	0.1 ± 0.2
	−Zn	0	0	0.6	0.2	0.3	0	3.1	0.7 ± 0.6
	−P	0.4	0.6	0.8	1.8	0.5	1.7	0	0.7 ± 0.5
Total	Complete	0.2	0.2	1.0	0.2	0.3	0.2	0.1	0.3 ± 0.2
	−Zn	0.2	0.5	1.5	1.2	1.3	0.4	3.8	1.4 ± 0.7
	−P	2.8	1.8	2.1	3.6	1.1	3.2	0.3	1.9 ± 0.9

"Total" includes oxalate, citrate, malate, tartrate, malonate, fumarate, maleate and succinate (in order of decreasing average concentration). Genotypes are presented in order of increasing Zn efficiency. Values for each genotype are means of three (oxalate and total) or six (citrate) replicates.
[1]Means ± SE; for citrate: $n = 42$; for other oxalate and total: $n = 21$.
[2]"0" means $< \pm 0.1$ nmol g^{-1}plant dw s^{-1}.

for all genotypes. However, further increases in planting density reduced shoot dry matter of tolerant genotypes (Jalmagna, M79, 507), whereas the highly intolerant genotype Dular showed further improvements. With a low rate of citrate (and total LMWOAs) exudation, Dular and IR74 may need the combined exudates of ten root systems to reach maximum Zn solubilisation and uptake and dry weight, whereas this can already be achieved at a density of five plants per hill for tolerant genotypes with higher exudation rates. Further increases in these genotypes led to reduced dry weight per plant probably due to a mutual shading effect.

The work of Kirk et al. (1999) shows that the exudation rates we found could have a large impact on Zn and P mobilisation. The citrate exudation rates for plants grown on a complete nutrient solution (Table 2) were higher than those reported by them. They showed that the increased rates they found at P stress (± 1.6 μmol g^{-1} root dw h^{-1}) can account for the relatively high capacity of rice to mobilise P from a P-deficient aerobic soil. Effects of exudation in a flooded soil, however, will be less due to fast diffusion. If exudation would be localised to parts of the root system, only, as was shown for white lupine and rape, then the impact would increase (Hoffland, 1992).

Increased exudation of LMWOAs as a consequence of both Zn and P deficiency adds to the concept of LMWOAs as a multiple stress response (Jones, 1998; Marschner, 1995; Raghothama, 2000). As far as we are aware this concept has not been confirmed before for both a macronutrient and a micronutrient deficiency within one plant species. It has been confirmed for two toxic metals (Nian et al., 2002), but here the stress response must be regulated differently from nutrient deficiency (cf. Dong et al., 2004), because the response shows within hours after exposure. Recently, Hajiboland et al. (2005) showed increased LMWOA accumulation in rice roots as a response to bicarbonate. It remains to be investigated whether mechanisms involved in increased exudation of LMWOAs as a response to P deficiency, including increased PEP carboxylase activity in either shoot (Hoffland et al., 1992) or root (Uhde Stone et al., 2003), are also involved in Zn deficiency-induced exudation. The differential response of individual genotypes to the two different nutrient deficiencies (Table 4) regarding the type of LMWOA exuded, suggests a genotype- and nutrient-specific regulation of biosynthesis, catabolism and transport downstream from PEP carboxylase.

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References

Aulakh M S, Wassmann R, Bueno C, Kreuzwieser J and Rennenberg H 2001 Characterization of root exudates at different growth stages of ten rice (*Oryza sativa* L.) cultivars. Plant Biol. 3, 139–148.

Dinkelaker B, Römheld V and Marschner H 1989 Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupine (*Lupinus albus* L.). Plant Cell Environ. 12, 285–292.

Doberman A and Fairhurst T 2000 Rice: Nutrient Disorders and Nutrient Management. IRRI & PPI & PPIC, Makati City & Singapore.

Dong D, Peng X and Yan X 2004 Organic acid exudation induced by phosphorus deficiency and/or aluminium toxicity in two contrasting soybean genotypes. Physiol. Plant. 122, 190–199.

Erenoglu B, Eker S, Cakmak I, Derici R and Römheld V 2000 Effect of iron and zinc deficiency on release of phytosiderophores in barley cultivars differing in zinc efficiency. J. Plant Nutr. 23, 1645–1656.

Gao X P, Zou C, Zhang F S, Van der Zee S E A T M and Hoffland E 2005 Tolerance to Zn deficiency in rice in relation with Zn uptake. Plant Soil, accepted.

Gardner W K, Barber D A and Parbery D G 1983 Acquisition of phosphorus by *Lupinus albus* III. Probable mechanism by which phosphorus movement in the soil root interface is enhanced. Plant Soil 70, 107–124.

Geelhoed J S, Hiemstra T and Van Riemsdijk W H 1998 Competitive interaction between phosphate and citrate on goethite. Environ. Sci. Technol. 32, 2119–2123.

Hacisalihoglu G and Kochian L V 2003 How do some plants tolerate low levels of soil zinc? Mechanisms of zinc efficiency in crop plants. New Phytol. 159, 341–350.

Hajiboland R, Yang X E and Römheld V 2003 Effects of bicarbonate and high pH on growth of Zn-efficient and Zn-inefficient genotypes of rice, wheat and rye. Plant Soil 250, 349–357.

Hajiboland R, Yang X E, Römheld V and Neumann G 2005 Effect of bicarbonate on elongation and distribution of organic acids in root and root zone of Zn-efficient and Zn-inefficient rice (*Oryza sativa* L.) genotypes. Environ. Exp. Bot. 54, 163–173.

Hoffland E 1992 Quantitative evaluation of the role of organic acid exudation in the mobilization of rock phosphate by rape. Plant Soil 140, 279–289.

Hoffland E, Findenegg G R and Nelemans J A 1989 Solubilization of rock phosphate by rape. II. Local root exudation of organic acids as a response to P-starvation. Plant Soil 113, 161–165.

Hoffland E, Van den Boogaard R, Nelemans J A and Findenegg G R 1992 Biosynthesis and root exudation of citric and malic acids in phosphate-starved rape plants. New Phytol. 122, 675–680.

Hopkins B G, Whitney D A, Lamond R E and Jolley V D 1998 Phytosiderophore release by sorghum, wheat, and corn under zinc deficiency. J. Plant Nutr. 21, 2623–2637.

Jones D L 1998 Organic acids in the rhizosphere. A critical review. Plant Soil 205, 25–44.

Kirk G J D and Bajita J B 1995 Root-induced iron oxidation, pH changes and zinc solubilization in the rhizosphere of lowland rice. New Phytol. 131, 129–137.

Kirk G J D, Santos E E and Findenegg G R 1999 Phosphate solubilization by organic anion excretion from rice (*Oryza sativa* L.) growing in aerobic soil. Plant Soil 211, 11–18.

Lipton G S, Blanchar R W and Blevins D G 1987 Citrate, malate and succinate concentrations in exudates from P-sufficient and P-stressed *Medicago sativa* L. seedlings. Plant Physiol. 85, 315–317.

Marschner H 1995 Mineral Nutrition of Higher Plants. Academic Press, San Diego etc 889 pp.

Neue H U, Quijano C, Senadhira D and Setter T 1998 Strategies for dealing with micronutrient disorders and salinity in lowland rice systems. Field Crops Res. 56, 139–155.

Nian H, Yang Z M, Ahn S J, Cheng Z J and Matsumoto H 2002 A comparative study on the aluminium- and copper-induced organic acid exudation from wheat roots. Physiol. Plant. 116, 328–335.

Quijano-Guerta C, Kirk G J D, Portugal A M, Bartolome V I and McLaren G C 2002 Tolerance of rice germplasm to zinc deficiency. Field Crops Res. 76, 123–130.

Raghothama K G 2000 Phosphorus acquisition; plant in the driver's seat!. Trends Plant Sci. 5, 412–413.

Rengel Z and Römheld V 2000 Root exudation and Fe uptake and transport in wheat genotypes differing in tolerance to Zn deficiency. Plant Soil 222, 25–34.

Rengel Z, Römheld V and Marschner H 1998 Uptake of zinc and iron by wheat genotypes differing in tolerance to zinc deficiency. J. Plant Physiol. 152, 433–438.

Uhde Stone C, Gilbert G, Johnson J M F, Litjens R, Zinn K E, Temple S J, Vance C P and Allan D L 2003 Acclimation of white lupin to phosphorus deficiency involves enhanced expression of genes related to organic acid metabolism. Plant Soil 248, 99–116.

Van Breemen N and Castro R U 1980 Zinc deficiency in wetland rice along a toposequence of hydromorphic soils in The Philippines. Plant Soil 57, 215–221.

Westergaard B, Hansen H C B and Borggaard O K 1998 Determination of anions in soil solutions by capillary zone electrophoresis. Analyst 123, 721–724.

Yang X, Römheld V, Marschner H and Barrow N J 1993 Effect of bicarbonate and root zone temperature on uptake of Zn, Fe, Mn and Cu by different rice cultivars (*Oryza sativa* L.) grown in calcareous soil. Plant Soil 155/156, 441–444.

Zhang X, Zhang F and Mao D 1998 Effect of iron plaque outside roots on nutrient uptake by rice (*Oryza sativa* L.). Zinc uptake by Fe-deficient rice. Plant Soil 202, 33–39.

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