Rhizosphere properties of rice genotypes as influenced by anoxia and availability of zinc and iron

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Abstract – The objective of this work was to study possible mechanisms involved in root-induced changes of rhizosphere physicochemical properties of rice genotypes, under anoxia and low supply of Zn and Fe. Two rice genotypes, including an upland and a lowland ones, were grown in hydroponic medium under adequate and low supply of Zn and Fe, with or without aeration. Anoxia increased shoot dry weight, root length and uptake of Zn and Fe in lowland Amol genotype, but reduced these parameters in upland Gasrol-Dashti genotype. The amount of oxygen released by roots was statistically higher in 'Amol'. The highest acidification potential of roots was observed in the lowland genotype under low supply of Zn, and in the upland genotype under Fe starvation. The highest oxalate (only organic acid detected) exudation from roots was observed in Zn and Fe deficient Gasrol-Dashti genotype. Zinc deficiency caused reduction of alcohol dehydrogenase and stimulation of lactate dehydrogenase activity, particularly in shoot. The ability to induce changes in the rhizosphere properties has a great contribution for the adaptation of both lowland and upland rice genotypes to specific soil conditions.

Index terms: *Oryza sativa*, alcohol dehydrogenase, alpha-naphthylamine oxidation, lactate dehydrogenase, lowland genotype, proton release, upland genotype.

Propriedades da rizosfera de genótipos de arroz submetidos à anoxia e a diferentes disponibilidades de zinco e ferro

Resumo – O objetivo deste trabalho foi estudar possíveis mecanismos envolvidos em mudanças nas propriedades físico-químicas da rizosfera, em genótipos de arroz sob anoxia e baixo suprimento de Zn e Fe. Dois genótipos de arroz, um de terras altas e outro de várzeas, foram cultivados em meio hidropônico, com suprimento adequado ou baixo de Zn e Fe, em condições anóxicas ou aeradas. A anoxia aumentou a massa de matéria seca da parte aérea, o comprimento de raízes e a absorção de Zn e Fe, no genótipo de várzeas Amol, mas reduziu esses parâmetros no genótipo de terras altas Gasrol-Dashti. A quantidade de oxigênio liberada pelas raízes foi superior no 'Amol'. Maior potencial de acidificação das raízes foi observado no genótipo de várzeas, sob baixo suprimento de Zn, e no genótipo de terras altas, sob baixo suprimento de Fe. A maior exsudação de oxalato ocorreu com o 'Gasrol-Dashti', com deficiência de Zn e Fe. A deficiência de Zn reduziu a atividade da álcool desidrogenase e estimulou a da lactato desidrogenase, especialmente na parte aérea. A habilidade em induzir mudanças em propriedades da rizosfera possui grande importância na adaptação a condições específicas do solo, em ambos os genótipos.

Termos para indexação: *Oryza sativa*, álcool desidrogenase, oxidação da alfa-naftilamina, lactato desidrogenase, várzea, extrusão de prótons, arroz de terras altas.

Introduction

Zinc deficiency is found to be a widespread phenomenon in lowland (Quijano-Guerta et al., 2002) and upland rice (Fageria & Baligar, 2005); and Fe deficiency is a common disorder of rice growing on well drained soils (Nerkar et al., 1984), whether these are neutral, calcareous or alkaline.

Most of the world's rice is cultivated under irrigated or rainfed lowland conditions. Under reductive conditions in flooded soils, the availability of Zn is extremely low, due to formation of Zn sulfide, which presents very low solubility, and adsorption or co-precipitation of Zn onto calcium carbonate particles, in the presence of a large amount of carbon dioxide. Together with nitrogen and phosphorus deficiency, Zn deficiency is now considered

the most widespread nutrient disorder in lowland rice (Quijano-Guerta et al., 2002).

The porous aerenchymatous tissues in rice can transport photosynthetically produced compounds or atmospheric oxygen from shoot to roots (Armstrong, 1969). Moreover, considerable amount of oxygen is released from roots, resulting in H^+ generation from oxidation processes and consequently solubilization of Zn in the rhizoplane, what can increase the availability of Zn to rice plants under flooding conditions (Kirk & Bajita, 1995).

Armstrong (1969) determined genotypic differences in oxygen diffusion from rice roots and related that to resistance to iron toxicity. It was also shown that roots of Zn-efficient rice genotypes have a greater oxidizing power and broader oxidizing zones around the root, than the inefficient ones (Yang et al., 1994). Oxidation power of rice roots comprises two components: oxygen release and enzymatic oxidation measured by alpha-naphthylamine (Ando et al., 1983).

In addition of rhizosphere acidification, exudation of low molecular weight organic anions into rhizosphere could significantly enhance Zn availability to plants (Marschner, 1998). Rice plants have been found to exude organic acids, when grown hydroponically or in soil (Hajiboland et al., 2005; Hoffland et al., 2006). It was suggested that the citrate exudation capacity of rice genotypes was related to their tolerance to Zn deficiency (Hajiboland et al., 2005). Organic acids can increase soil Zn availability in two ways, they are probably exuded both with protons and as counter ions and, consequently, reduce rhizosphere pH. In addition, the anions can chelate Zn and increase Zn solubility (Jones, 1994).

Because of water constraints, rice production worldwide, especially in Asia, is now in transition from the traditional high water consuming lowland rice cultivation, with flooded fields, to a new cultivation system of aerobic upland rice (Gao, 2007). Many factors that determine bioavailability of Zn are expected to change after a shift to aerobic cultivation. Recent studies showed that cultivation of rice, on drained calcareous soils, increases Zn deficiency problems in rice (Gao, 2007). Iron deficiency is also expected to be a limiting factor for rice production under aerobic conditions (Fageria et al., 1997). Increased nitrification in aerobic soils may cause plants to take up NO_3^- instead of NH_4^+, which also causes the rhizosphere pH to increase (Gao, 2007).

Alcohol dehydrogenase (ADH) is the major terminal enzyme of fermentation in plants, and it is responsible for recycling of NAD during anoxia. Since ADH is a Zn containing enzyme, its activity is likely important in the response of rice genotypes to flooding conditions. Moore Júnior & Patrick (1988) reported that, in response to low Zn supply, root ADH activity decreased in rice plants grown in flooded soil, which was accompanied by a reduction in plant growth and Zn concentration. It has been suggested that ethanolic fermentation permits tight cytoplasmic pH regulation, thus preventing acidosis from lactic fermentation catalyzed by lactate dehydrogenase (LDH) (Roberts et al., 1985). There is evidence that rice cultivars with higher tolerance to prolonged submergence show significantly faster rates of alcoholic fermentation than the intolerant ones (Huang et al., 2003).

It seems likely that, genotypic differences between lowland and upland genotypes, regarding tolerance to flooding conditions, are at least partially attributable to root-induced changes in the physicochemical properties of rhizosphere. Moreover, because of different availability of nutrients under these two cultivation systems, particularly of Zn (Gao, 2007) and Fe (Fageria et al., 1997), genotypic differences are also expected for nutrient acquisition properties.

The objective of this work was to investigate possible mechanisms involved in root-induced changes of rhizosphere physicochemical properties in two contrasting rice genotypes.

Materials and Methods

Two genotypes of rice (*Oryza sativa* L.) were used in this work: Amol, a lowland genotype from the North of Iran; and Gasrol-Dashti, an upland genotype from the South of the country. Seeds were provided by the Rice Research Center and the Agricultural Research Center, for 'Amol' and 'Dashti', respectively.

Plants were cultivated in a growth chamber under Zn and Fe deficiency conditions, in the nutrient solution, either with or without aeration. The temperature regime was 25–18°C day-night, with 14–10 hours light-dark period, and relative humidity of 70–80%, under photon flux density of 350 μmol m^{-2} s^{-1}. Rice seeds germinated in the dark on filter paper soaked with saturated $CaSO_4$ solution. The germinated seeds were transferred onto nylon nets, which were suspended and flooded with a 0.02 mM $CaSO_4$ solution in a 10 L container, for 2 days.

Thereafter, plants were pre-cultured in 25 and 50% ionic strength nutrient solutions (Yoshida et al., 1972), for 2 and 3 days, respectively. Subsequently, 13-day-old seedlings with similar sizes were selected and transferred to 2 L dark plastic containers, and were pre-cultured for another 3 days in 50% ionic strength nutrient solution (pH 6.8). Concentration of Zn in the pre-culture medium of Zn experiment were 0.5 (adequate) and <0.08 μM (low), and Fe concentrations were 100 (adequate) and 10 μM (low) in the Fe experiment.

In the Fe experiment, sixteen-day-old plants were treated either with adequate (100 μM) or low (zero Fe) concentration in the conventional rice nutrient solution (pH 6.8) (Yoshida et al., 1972). Plants were grown only for 21 days, because of severe Fe deficiency in this experiment. For comparison of growth under anoxia and aerobic conditions, plants were cultivated simultaneously in aerated and nonaerated nutrient solutions. Nutrient solutions were completely changed every 7 days, and pH was adjusted every day. Release of proton and organic acids from roots, and the oxidation and oxygenation power of roots were determined in plants grown for 21 days.

The Zn experiment was conducted with sixteen-day-old plants, which were transferred from pre-culture medium to chelator-buffered nutrient solution (Yang et al., 1994). Zinc treatments consisted of two levels of added $ZnSO_4$ at 2 and 20 μM (free Zn^{2+} activities were 12 and 130 pM). Plants were grown for 42 days, and their growth and Zn uptake were determined. For obtaining comparable results with Fe experiment, the determinations were performed in plants grown only for 21 days.

After the end of the treatments period, plants were washed with double-distilled water, divided into shoots and roots, weighted and blotted dry on filter paper, and dried at 70°C for 2 days, in order to determine shoot and root dry weight. Another group of plants were used for determination of root length (Tennant, 1975).

For determination of Zn and Fe content of plant tissues, oven-dried samples were ashed in a muffle furnace at 550°C for 8 hours; thereafter, samples were resuspended in 2 mL 10% HCl made up to volume by double-distilled water. Zinc and Fe concentrations were determined through atomic absorption spectrophotometry (Shimadzu, AA 6500).

Measurement of oxygen release from roots was conducted using an oxygen electrode (Consort, Z921). The root system of an intact plant was placed in a glass container with 70 mL oxygen-free solution containing 2 mM $Ca(NO_3)_2$. Plants were fixed tightly in 1 cm holes using parafilm. Release of oxygen was measured for 10 min at 22°C, after stirring, and by following the linear increase of O_2 concentration over time, between the third and eight minutes of the measurement period. Four replicates, taken from plants of four pots, were used for each measurement. Thereafter, fresh weight of the root was determined. Respiration rate was expressed as mg O_2 release g^{-1} root fresh weight min^{-1} (Ando et al., 1983), slightly modified.

For measurement of oxidation power of roots, intact plants were immersed in 50 mL of 20 ppm alpha-naphthylamine (alpha-NA) test solution for 10 min to exclude initial rapid absorption of alpha-NA by roots. The intact roots were transferred to another 50 mL of 20 ppm alpha-NA solution, which were maintained at 25±1°C, and incubated up to four hours. After 2 and 4 hours of incubation, 2 mL of the alpha-NA sample solution were pipetted out, reacted with 10 mL of 0.1% sulfanilic acid (in 3% acetic acid) and, then, with 2 mL of 50 ppm $NaNO_2$. After dilution to 25 mL with distilled water, the absorbance of the colored solution was determined at 530 nm. The decrease in alpha-NA, during incubation, was calculated as amount of alpha-NA oxidized by the roots in a given time (Ando et al., 1983). Data obtained after 2 and 4 hours of incubation have similar trend of differences considering treatment and genotype, but the former values were slightly lower than the latter ones; only the oxidation data after 4 hours of root incubation was reported.

For a quantitative demonstration of changes in the pH of medium, after 14 days of growth in the treatment solution, plants were let to grow for one week further, without pH adjustment. Simultaneously with nutrient solution change, plants were transferred to the nonchelator buffered (conventional) nutrient solution (Youshida et al., 1972), adjusted at pH 6.5 only in the first day. The following one-week of growth was without pH adjustment, and pH was monitored daily. The calculation of H^+ release was performed from changes in the pH values, considering root fresh weight and volume of nutrient solution.

Organic acids exuded from roots were collected using filter paper method (Hajiboland et al., 2005). Roots were rinsed with double distilled water at root temperature (23°C), then, they were spread on a plate covered with polyethylene foil, which was repeatedly sprayed with distilled water. Five to seven axes of roots with similar size (25–30 cm), from each replicate plant, were gently

isolated from the rest of the root system without cutting. Root axes were incubated between two pieces of moist filter paper with 1.5×30 cm. Filter papers had been previously washed with methanol and distilled water for 3 and 2 times, respectively, and dried at room temperature. The rest of roots was covered with moist paper, and the plate was covered with a lid. Three hours later, the filter papers were taken from root surfaces, cut to small pieces and placed in centrifugal vials. After addition of 3 mL deionized water, exudate samples were centrifuged at 10,000 *g* for 5 min at 4°C. The corresponding root segments were cut and weighted, both supernatant of exudate samples and root segments were immediately frozen in liquid N_2 and stored at -20°C until analysis.

The frozen root samples were ground in liquid nitrogen and then extracted in 10 mL of H_3PO_3 (5%) per each gram of fresh root material. Samples were centrifuged at 10,000 *g* for 10 min, and the supernatant was used for organic acids analysis. Organic acids in the exudates samples and roots were determined using HPLC (Knauer). Samples were injected onto the column, and 18 mM KH_2PO_4 (pH 2.1, adjusted by H_3PO_3) were used for isocratic elution with a flow rate of 0.5 mL min^{-1} at 28°C and UV detection at 215 nm (Hajiboland, et al., 2005). Prior to HPLC injection, the samples were diluted by addition of appropriate volumes of eluant.

For the determination of alcohol dehydrogenase (ADH; EC 1.1.1.1) activity, roots were rinsed with double distilled water and, after drying on filter paper, were frozen and stored in liquid N_2 until assaying. Determination of the enzyme activity was carried out according to Pedrazzini & McKee (1984). Samples were ground under liquid N_2 to a fine powder, then homogenized with 50 mM cold HEPES buffer containing 5 mM $MgCl_2$, 2 mM cysteine hydrochloride and 2% (W/V) insoluble polyvinylpolypyrrolidone (PVPP) at pH 7.8. After centrifugation at 10,000 *g* for 10 min, the supernatant was used for determination of enzyme activity. The assay solution (pH 8) contained 14 mM HEPES, 5.4 mM $MgCl_2$, 0.13 mM NADH. The reaction was started by addition of 4 mM acetaldehyde at 30°C. Activity was monitored as disappearance of NADH at 340 nm for 3 min.

The lactate dehydrogenase activity (LDH; EC 1.1.1.27) was assayed according to Vassault (1987). Ground root samples were extracted in Tris-NaCl solution (81.3 mM Tris, 203.3 mM NaCl, pH 7.2). The assay solution contained Tris-NaCl solution and 0.24 mM NADH. The reaction was started by addition of 9.76 mM pyruvate at 30°C, and the activity was monitored as disappearance of NADH at 340 nm.

Experiments were carried out under a completely randomized design in a 2x2x2 factorial array with four replications. The factors were two levels of Zn (or Fe) supply, two levels of aeration conditions and two genotypes. Statistical analyses were carried out using Sigma Stat (3.02), and means were compared by Tukey test, at 5% probability.

Results and Discussion

Low supply of Zn caused significant reduction of shoot dry weight (DW) and root length with an obvious genotypic effect (Table 1). Shoot DW of 'Amol' was

Table 1. Shoot dry weight and root length of two rice genotypes (*Oryza sativa* cvs. Amol and Dashti) grown for 42 (Zn experiment) or 21 (Fe experiment) days, either with adequate or low Zn and Fe supply, in aerated or nonaerated nutrient solution[(1)].

Genotype	Nutrient availability	Shoot dry weight (mg $plant^{-1}$)		Root length (cm $plant^{-1}$)	
		Aerated	Nonaerated	Aerated	Nonaerated
			Zn experiment		
Amol	+Zn	631±45b	797±25a	872±39b	1,312±34a
	-Zn	390±13d	492±15c	386±20d	655±53c
Dashti	+Zn	521±38a	413±41b	1,083±67a	592±96b
	-Zn	172±25c	107±5d	339±26c	229±42c
			Fe experiment		
Amol	+Fe	106±15b	162±12a	252±15b	316±27a
	-Fe	33±4c	53±3c	21±2c	31±7c
Dashti	+Fe	119±4a	80±7b	315±34a	175±13b
	-Fe	57±5c	36±2d	42±4c	28±5c

[(1)]Means of each genotype within each growth parameter, in a same experiment, followed by equal letters, do not differ by Tukey test, at 5% probability; reported values represent the means±SD.

reduced about 38%, while that of 'Dashti' was inhibited up to 67%. Similar differential response of genotypes to low Zn stress was observed for root length. In contrast, 'Amol' was more susceptible to Fe deficiency (with 69% reduction of shoot DW) than 'Dashti' (52%). A high susceptibility to Fe deficiency in this genotype is likely the result of low efficiency in the uptake, due to high available Fe in flooded soils (Marschner, 1995).

Anoxia conditions influenced plant growth differentially. Shoot DW and root length of 'Amol' were stimulated up to 26–53% when grown in nonaerated nutrient solution. The opposite effect was observed for 'Dashti' (Table 1). Greater root length for nutrients acquisition is particularly important for Zn, because of higher dependency to interception mechanism for the uptake of this nutrient (Barber, 1995).

Significant interactions were observed between the three factors. A great difference between genotypes in their response to Zn or Fe supply and anoxia is the main cause of these significant interaction effects.

Anoxia caused reduction of Zn and Fe uptake in 'Dashti' up to 15 and 20%, respectively, but increased them 17 and 50% in 'Amol' (Figure 1). For soil grown plants, not only lower capacity for nutrient absorption is expected, due to lower surface area of the root system, but also lower efficiency in nutrient acquisition *per se*. These factors account for lower performance under unfavorable soil conditions in terms of oxygen availability. Reduced production of energy for growth and maintenance processes, during submergence (Ram et al., 2002), could be an important cause of nutrients uptake reduction, under anoxia, for Dashti genotype. Reduction of uptake under aerated conditions in lowland genotype, in turn, could be attributed to production of more reactive oxygen species, such as superoxide radicals, in aerated plants. Reactive oxygen species attack phospholipids and transporter proteins (Kappus, 1985) and could strongly alter nutrient transport via membranes.

Zinc starvation resulted in an increase in oxygen release from roots; this effect was significant in aerated

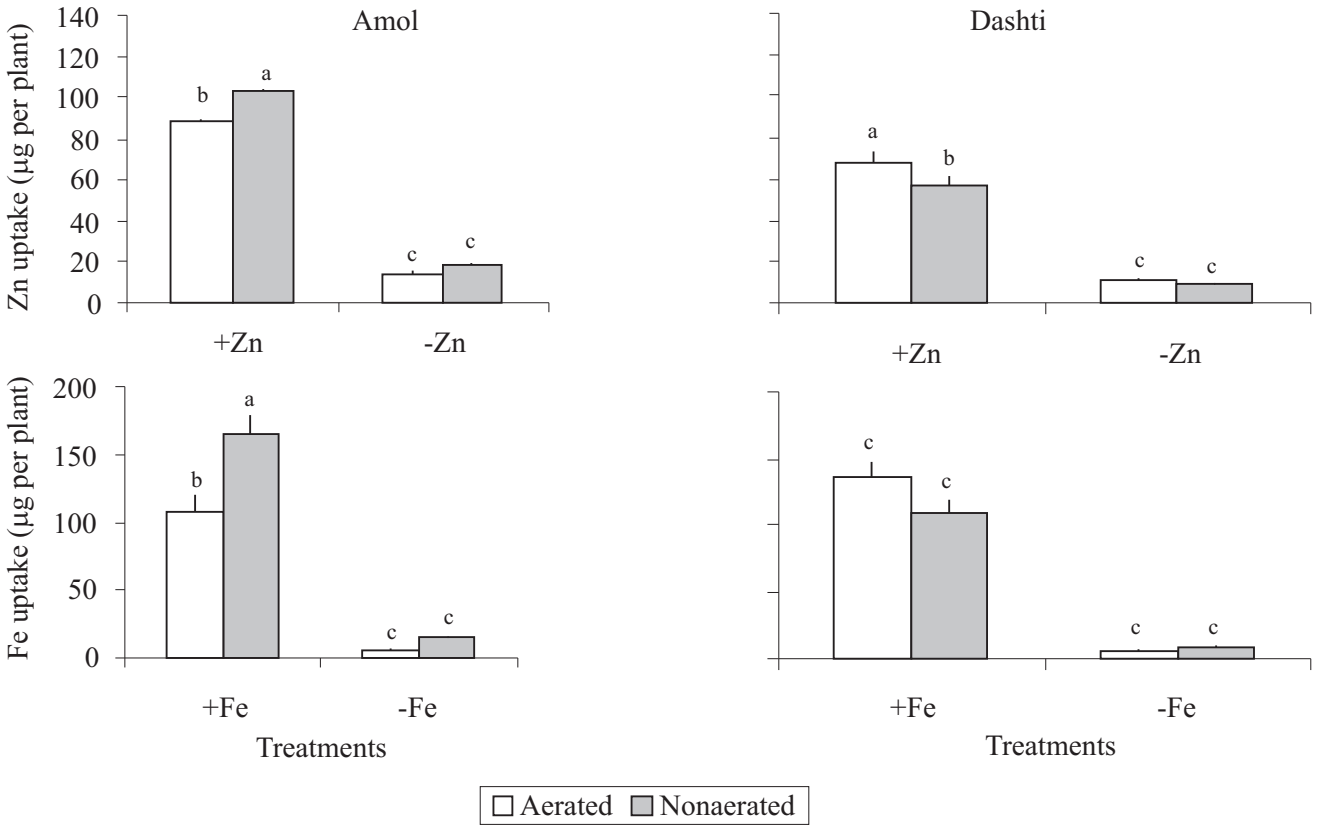

Figure 1. Zinc and Fe uptake (µg per plant) by two rice genotypes (*Oryza sativa* cvs. Amol and Dashti), grown for 42 (Zn experiment) or 21 (Fe experiment) days either with adequate or low Zn and Fe supply, in aerated or nonaerated nutrient solution. Bars indicate the mean±SD of four repetitions. Bars indicated by equal letters are not significantly different ($p<0.05$).

Amol and in nonaerated Dashti genotypes (Figure 2). Iron deficiency had a distinct increasing effect on oxygenation power of roots only in 'Dashti' under anoxia. Absolute amounts of oxygen released from roots were distinctly higher in 'Amol' than in 'Dashti'. A slight effect of anoxic conditions on increasing oxygen release by roots was observed also by Ando et al. (1983), and has been considered an adaptation to the reducing conditions of root environment. Higher oxygen release by roots of 'Amol' compared with 'Dashti', irrespective to the nutritional status of plants, is probably the result of formation of more developed aerenchymatous tissues, in the former genotype. However, the cause of slight or significant effect of Zn and Fe deficiency on induction of more release of oxygen by roots is not clear. Likely, Zn deficient plants developed more aerenchymatous tissues than the control ones. Nitrogen and Ca deficiency was reported to reduce oxygen release by rice roots, and K deficiency had no effect (Ando et al., 1983). Size, structure and metabolic activity of shoot and root may affect oxygen release rate. For instance, a longer root may have a lower oxygen release rate because more oxygen is consumed by the tissue along the pathway of transport (Ando et al., 1983). This explanation may apply to a higher oxygen release of Zn and Fe deficient plants with shorter root length, as well as to different effect of

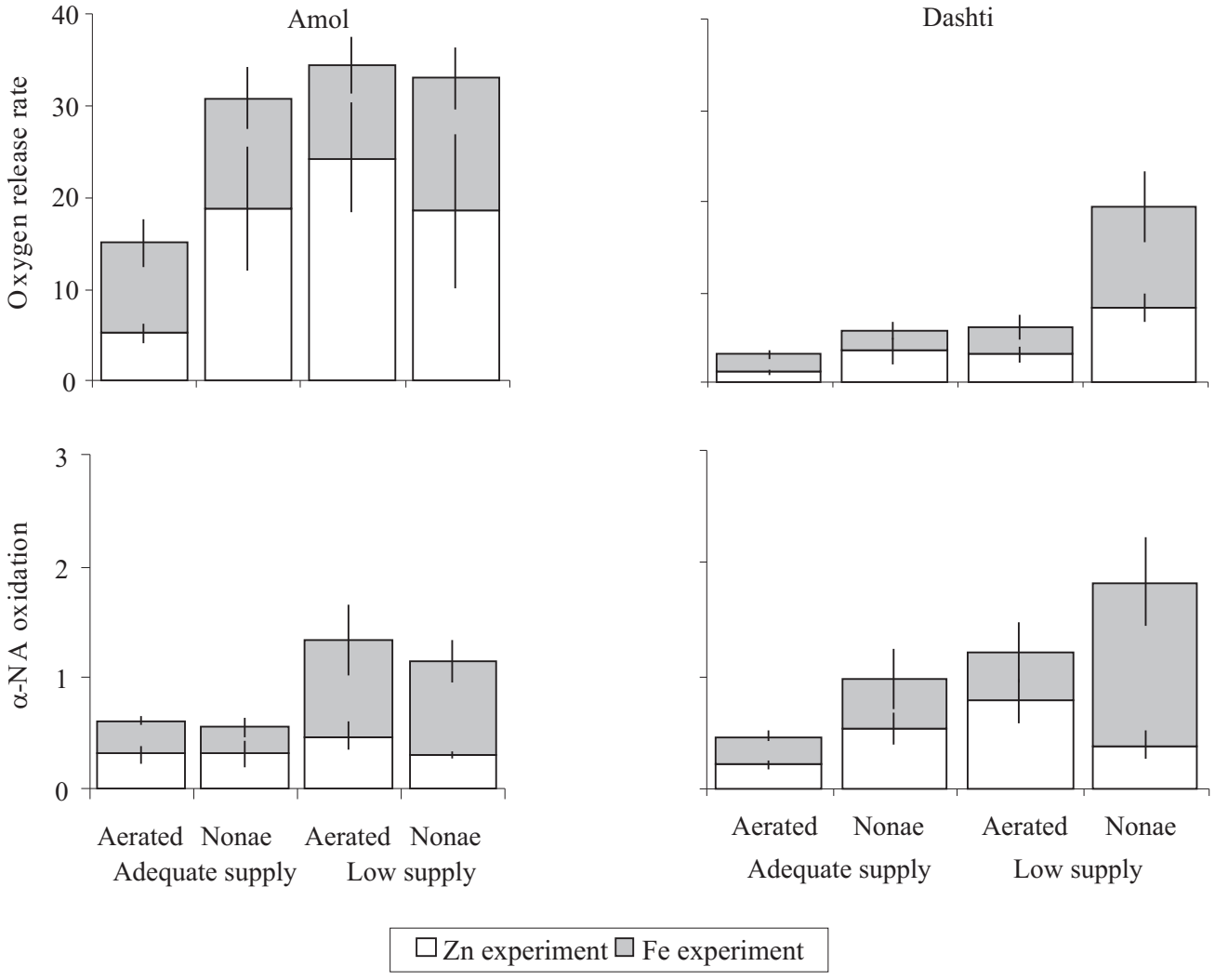

Figure 2. Oxidation of alpha-naphthylamine (α-NA) (mg g^{-1} root FW) and rate of oxygen release (μg O_2 g^{-1} root FW min^{-1}) by roots of two rice genotypes (*Oryza sativa* cvs. Amol and Dashti), grown for 42 (Zn experiment) or 21 (Fe experiment) days, either with adequate or low Zn and Fe supply, in aerated or nonaerated (Nonae) nutrient solution. Bars indicate the mean±SD of four repetitions.

anoxia, depending on genotype. Higher O_2 release could led to higher H^+ generation, in flooded soil, and to Zn bioavailability increase for plants (Kirk & Bajita, 1995).

Low Zn and anoxia did not affect oxidation power of roots in Amol genotype, which was increased up to 2.9 fold by Fe deficiency (Figure 2). In contrast to 'Amol', both low supply of Zn and Fe and anoxia affected oxidation power of roots in Dashti genotype. Zinc starvation caused induction of root oxidation only in aerated plants; and effect of Fe deficiency was observed only under anoxia. Nitrogen deficiency was reported to decrease alpha-NA oxidation by rice roots, P and Ca deficiency causes alpha-NA oxidation increase, while Mg deficiency has no effect (Ando et al., 1983). Since alpha-NA oxidation is closely related to respiration (Ando et al., 1983), its induction could be regarded as a consequence of increased respiration of roots. An increased root respiration was observed for rice plants under low Zn supply (Hajiboland, 2000).

Both genotypes strongly lowered pH of nutrient solution, with clear genotypic differences (Table 2). Low Zn supply had distinct stimulating effect on proton release by roots; Zn deficient Amol genotype showed much greater acidification potential of rhizosphere compared to 'Dashti'. Rhizosphere of rice roots becomes greatly acidified, as a result of H^+ generation during Fe^{2+} oxidation by root, released O_2 and an excess amount of cation (mainly NH_4^+) versus anion absorption (Kirk & Bajita, 1995). All these factors likely contributed in the release of proton by roots in our hydroponic experiment. The rate of proton release (10–40 μmol H^+ g^{-1} RFW d^{-1}) was slightly lower than reported amounts for phosphorus deficient rice (Kirk & Du, 1997). High pH reduces the availability of Zn in soil (Marschner, 1995) and root-mediated processes that can lower the rhizosphere pH and increase plant Zn availability by solubilizing Zn from organic and inorganic soil solid phase (Hacisalihoglu & Kochian, 2003). A great acidification potential of roots in 'Amol' is most likely an important cause of this genotype adaptation to low Zn conditions in flooded soils.

Rice is a strategy II plant and produces and secretes phytosiderophores under conditions of Fe deficiency, however, in lower amounts than other graminaceous crops such as barley (Mori et al., 1991). In addition of a strategy II Fe uptake system, rice plants, similarly as strategy I plants, have a direct Fe^{2+} uptake system that uses the Fe^{2+} membrane transporter (Ishimaru et al., 2006). The rice genome contains 19 putative *OsYSL* genes, which transport Fe^{2+}-nicotianamine but not Fe^{3+}-phytosiderophores (Koike et al., 2004). It was observed that transcription of *OsYSL2* gene is induced specifically in Fe-deficient rice plants (Koike et al., 2004). Proton release by roots, as one of the important components of strategy I for Fe acquisition, is characteristic for nongramineous plants (Marschner, 1995). Another similarity of rice with strategy I plants, and documented in this work, is the release of considerable amounts of proton under Fe starvation.

Root content of oxalate in Zn and Fe deficient plants was slightly or significantly higher than in sufficient ones (Table 3). However, considerable amount of oxalate was released from roots under deficiency conditions. The highest oxalate exudation from roots was observed in 'Dashti' under Zn and Fe starvation, which suggests that release of organic acids would have a great contribution in mobilization of insoluble Zn and Fe sources in soil. It was shown that increased malate concentrations in soil solution can increase Zn bioavailability; and genotypic differences in Zn uptake of aerobic rice is partly explained for the plant capacity to exude malate into the rhizosphere (Gao, 2007). The rate of oxalate release (210–740 pmol g^{-1} RFW s^{-1}) was in the reported range for malate and citrate in other rice genotypes (Hajiboland et al., 2005). However, we could not detect measurable amounts of any other organic acid than oxalate, either in root extract or exudates, likely because of the use of genotypes different from the ones used by other authors.

Zinc deficiency caused up to 63–73% reduction of ADH activity in both tested genotypes, and its effect was more prominent in shoot than in root (Table 4). In

Table 2. Amount of proton (μmol H^+ g^{-1} RFW) released from roots of two rice genotypes (*Oryza sativa* cvs. Amol and Dashti), during one week of growth in nutrient solution, either with adequate or low Zn and Fe supply, in aerated or nonaerated nutrient solution, without daily pH adjustment[1].

Genotype	Nutrient availability	Aerated	Non-aerated
		Zn experiment	
Amol	+Zn	-[2]	4.3±0.27b
	-Zn	-	274.2±17.16a
Dashti	+Zn	-	1.0±0.05b
	-Zn	-	2.0±0.14b
		Fe experiment	
Amol	+Fe	6.9±0.52e	8.9±0.45e
	-Fe	6.9±0.46e	39.2±2.31c
Dashti	+Fe	18.8±2.31d	18.9±1.98d
	-Fe	132.4±10.22a	60.1±3.78b

[1]Means followed by equal letters, in each experiment, do not differ by Tukey test, at 5% probability; reported values represent the means±SD. [2]Not determined.

contrast, activity of LDH in shoot was stimulated by low Zn supply in both genotypes. Similarly to Zn, Fe deficiency exerted an inhibiting effect on ADH activity, but Fe starvation had no obvious effect on LDH. Alcohol dehydrogenase is a Zn-containing enzyme, and reduction of its activity in low Zn plants is expected. However, the effect of Fe deficiency on ADH activity could be considered an indirect effect, such as the shortage of substrates (glucose) as a consequence of strong reduction of photosynthesis in Fe–deficient plants (Marschner, 1995). Reduction of root ADH activity was reported in relation to Zn-deficient rice plants grown in flooded soil, which was accompanied by reduction of plants growth (Moore Júnior & Patrick Júnior, 1988). Submergence can shift aerobic respiration to the less efficient anaerobic fermentation as the main source of

Table 3. Root concentration of oxalate and rate of release into rhizosphere by two rice genotypes (*Oryza sativa* cvs. Amol and Dashti), grown either with adequate or low Zn and Fe supply, in aerated or nonaerated nutrient solution(1).

Genotype	Nutrient availability	Oxalate content (mg g^{-1} RFW)		Oxalate exudation rate (µg g^{-1} RFW 3 h^{-1})	
		Aerated	Nonaerated	Aerated	Nonaerated
			Zn experiment		
Amol	+Zn	24±3.9a	23±3.3ab	209±32b	204±54ab
	-Zn	17±1.1b	18±3.9ab	337±81a	297±29ab
Dashti	+Zn	18±4.1a	19±2.9a	251±32b	377±110b
	-Zn	12±1.6a	11±5.4a	339±104b	687±176a
			Fe experiment		
Amol	+Fe	27±2.2a	22±0.4b	312±64a	214±135a
	-Fe	21±3.9b	11±1.4c	357±94a	278±87a
Dashti	+Fe	22±3.2ab	27±0.3a	225±59b	251±81b
	-Fe	13±2.8b	17±2.2b	344±82b	532±77a

(1)Means of each genotype within each parameter, in a same experiment, followed by equal letters, do not differ by Tukey test, at 5% of probability; reported values represent the means±SD; n = 4.

Table 4. Activity of alcohol dehydrogenase (ADH) and lactate dehydrogenase (LDH) (nmol NADH mg^{-1} protein min^{-1}) in two rice genotypes (*Oryza sativa* cvs. Amol and Dashti), grown either with adequate or low Zn and Fe supply, in aerated or nonaerated nutrient solution (1).

Genotype	Nutrient availability	Root		Shoot	
		Aerated	Non-aerated	Aerated	Non-aerated
			ADH		
Amol	+Zn	51.9±15.5a	53.1±6.3a	54.3±10.2a	44.7±15.9a
	-Zn	39.1±8.0a	38.4±7.6a	14.4±2.9b	10.7±2.7b
Dashti	+Zn	57.9±7.6a	54.4±8.1a	51.4±5.2a	36.7±7.2b
	-Zn	42.1±10.3a	45.1±4.7a	18.9±5.8c	16.1±4.6c
			LDH		
Amol	+Zn	9.38±0.92b	9.49±0.65b	8.39±0.33b	7.57±0.99b
	-Zn	7.79±1.20b	11.53±0.65a	17.00±1.51a	17.48±1.30a
Dashti	+Zn	9.99±1.79a	7.35±1.33a	12.70±1.86c	14.98±2.97c
	-Zn	7.74±1.09a	9.97±4.54a	29.97±1.39a	22.89±3.58b
			ADH		
Amol	+Fe	27.6±10.8ab	47.0±7.7a	57.5±5.9a	44.6±0.5b
	-Fe	13.5±4.1b	13.8±5.6b	38.1±7.4b	28.8±1.9c
Dashti	+Fe	48.8±9.1a	39.4±6.4a	38.1±8.1a	32.9±2.8a
	-Fe	20.9±7.4b	14.2±4.2b	27.1±3.9a	25.1±7.3a
			LDH		
Amol	+Fe	7.63±1.40a	8.55±1.08a	14.71±0.44a	15.98±2.49a
	-Fe	7.66±1.17a	8.07±1.34a	16.16±2.96a	15.89±1.58a
Dashti	+Fe	6.36±1.13ab	7.98±0.30b	12.46±4.61a	12.20±2.62a
	-Fe	5.29±1.23b	5.04±0.90b	10.07±2.74a	9.81±2.67a

(1)Means of each genotype within each plant organ, followed by equal letters, do not differ by Tukey, test at 5% probability; reported values represent the means±SD.

energy production (Ram et al., 2002). The efficiency of this pathway depends on continued supply of substrates (glucose) and on the two key enzyme, alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC). Thus, increased alcoholic fermentation is one way to alleviate the adverse effect of anoxia on reduced production of energy for growth and maintenance of processes during submergence. The importance of alcoholic fermentation for growth and survival of rice has been well emphasized (Ram et al., 2002). Anoxic conditions showed no considerable effect on ADH and LDH activity. A direct involvement of ADH and LDH in the resistance of maize plants to anoxia has not been confirmed (Xia & Saglio, 1992).

Conclusions

1. Higher acidification potential of roots in lowland genotype, under Zn deficiency conditions, is an important factor in adaptation to soils with sparingly available Zn; in contrast, release of oxalate under low supply of Zn is a mechanism for adaptation in upland genotype.

2. Release of considerable amount of proton in response to low Fe supply, in upland genotype, shows that this genotype uses a mixed strategy for Fe acquisition.

3. Reduction of alcohol dehydrogenase in shoot of low Zn plants, accompanied by increased lactate dehydrogenase activity, demonstrates a shift from alcoholic to lactic fermentation in shoot of Zn starved plants.

Acknowledgements

To Dr. H. Nazemieh, for providing facilities for determination of organic acids by HPLC.

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Received on December 4, 2007 and accepted on April 25, 2008