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ARTICLE

Growth, gas exchange and function of antioxidant defense system in two contrasting rice genotypes under Zn and Fe deficiency and hypoxia

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ABSTRACT For study of underlying physiological mechanisms for genotypic differences in tolerance to Zn and Fe deficiency and hypoxia, two contrasting rice genotypes (*Oryza sativa* L. cv. Amol and Dashti) were studied in nutrient solution with or without aeration. Growth of the lowland genotype (Amol) was significantly improved in nutrient solution without aeration, the opposite was observed for upland genotype (Dashti). Tolerance of Amol to low Zn was higher than Dashti, in contrast the former genotype was more susceptible to Fe deficiency. Photochemistry of leaves was affected strongly by Fe but not Zn deficiency. Low supply of Zn and Fe impaired photosynthetic capacity of plants mainly via stomatal limitation and the amount of reduction in net assimilation rate correlated well with differential growth reduction under Zn and Fe deficiency stress. Under hypoxia, plants had lower stomatal conductance and transpiration rate leading to improved photosynthetic water use efficiency. Activity of ascorbate peroxidase (APX) and guaiacol peroxidase (POD) induced by low Zn supply, but low Fe caused reduction of APX, CAT and POD. Activity of SOD decreased in low Zn plants, but increased in plants suffered from Fe deficiency. Increased APX activity in response to hypoxic conditions in Amol was associated with higher tolerance in this genotype, in contrast POD activity only monitored stress conditions without any protecting role. A close correlation was observed between accumulation of $O_2^{\cdot-}$ and differential sensitivity of genotypes to hypoxia. **Acta Biol Szeged 52(2):283-294 (2008)**

KEY WORDS

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lowland rice
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upland rice

Rice culture is divided into two broad groups, upland and lowland culture. Upland rice refers to rice grown on both flat and sloping fields that are prepared and seeded under dryland conditions and relies entirely on rainfall or irrigation depending on the amount of precipitation. Flooded rice, known also as lowland or waterlogged rice, is grown on flatland in flooded soils (Fageria et al. 1997).

In submerged or flooded soils, limited oxygen availability and altered chemical reactions that involve in the nutrients availability e.g. Zn and Fe for plants, creates conditions markedly different from those of drained soils. Because of water constraints, rice production is now in transition from the traditional high water consuming lowland rice cultivation on flooded fields to a new cultivation system of aerobic rice (Gao et al. 2005). Aerobic rice is grown as a dry field crop in irrigated but non-flooded soils (Bouman et al. 2005). Zinc deficiency was reported for both upland (Fageria et al. 1997; Fageria and Baligar 2005) and lowland rice (Yang et al. 1994a; Hajiboland et al. 2003), however, Fe deficiency is a common disorder of rice growing on well drained (aerobic) soils (Nerkar et al. 1984), whether these are neutral, calcareous or alkaline.

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Hypoxic and anoxic conditions maintain transition metal ions in a more or less reduced state and induce formation of reactive oxygen species (ROS). The same conditions can arise within submerged plants (Hendry and Brocklebank 1985). Therefore, hypoxia survival may depend on the capacity of plant tissues to counterattack reactive oxygen species and limit damages.

One of the primary effects of these molecular species in cells is the peroxidation of membranes forming toxic products such as malondialdehyde (MDA; Kappus 1985). Plants have evolved various protective mechanisms to eliminate or reduce ROS. Enzymatic antioxidant system, which is one of the protective mechanisms including superoxide dismutase (SOD) are located in various cell compartments and catalyse the disproportion of two $O_2^{\cdot-}$ radicals to H_2O_2 and O_2 (Salin 1987). H_2O_2 is eliminated by various antioxidant enzymes such as catalase (CAT) and peroxidases (POD) converting H_2O_2 to water (Salin 1987). Ascorbate peroxidase (APX) eliminates peroxides by converting ascorbic acid to dehydroascorbate (Asada 1992). Ascorbate peroxidase and glutathione reductase (GR) are important components of the ascorbate-glutathione cycle responsible for the removal of H_2O_2 in different cellular compartments (Jiménez et al. 1997).

Activity of enzymes containing Zn or Fe as a catalytic or structural component is expected to be changed substantially in plants supplied with inadequate amounts of these nutrients. Among enzymes of antioxidant defense system, SOD contains Zn (Cu/Zn SOD isozyme) or iron (Fe-SOD isozyme in chloroplasts); iron contributes also as heme group in the structure of H_2O_2 scavenging enzymes including APX, POD and CAT (Marschner 1995). Therefore, plant growth under inadequate nutrients supply may be influenced *per se* by reduction in the capacity of antioxidant defense system.

Changes in chlorophyll fluorescence emissions are indications of changes in photosynthetic activity and the state of Photosystem II (Maxwell and Johnson 2000). The flow of electrons through PSII is indicative of the overall rate of photosynthesis and is an estimation of photosynthetic performance. It is plausible that not only low supply of Fe because of its involvement in electron transport and chlorophyll synthesis but also of Zn concerning its role for maintenance of integrity of membranes and protection of lipids and proteins against oxidative damage (Marschner 1995), may affect the photochemistry of leaves and inhibit biophysical processes of photosynthesis. Due to predominance of polyunsaturated fatty acids in thylakoid lipids (Gounaries et al. 1986), photosynthetic membranes in chloroplasts could be considered the most susceptible structures in plants grown under conditions of oxidative stresses.

Water availability differs greatly under flooded compared with non-flooded conditions. Therefore, plant strategies for water economy may be changed in transition from flooded to aerobic conditions, and every change via modification of stomatal conductance affects in turn photosynthetic CO_2 fixation. Recent studies showed that the water productivity (crop yield/water consumptively used in evapotranspiration) of rice under aerobic conditions is 32-88% higher than that under flooded conditions (Bouman et al. 2005).

In Iran, rice is the second important food crop after wheat and is cultivated mainly under flooded conditions in the north of the country. In south and central Iran, however, increasing water scarcity problem causes relatively long term non-submerged conditions during irrigation intervals in the field. Genotypes selected for higher tolerance to alternate submerged and non-submerged conditions, are now being used for upland cultivation. It is expected that, genotypes adapted and bred for one of these cultivation systems, have different tolerance to Zn and Fe deficiency and particularly hypoxia. Currently, aerobic rice varieties are developed by crossing lowland with upland varieties (Bouman et al. 2005).

Physiological basis of differences between traditional lowland genotypes with new genotypes with lower susceptibility to non-flooded conditions and its consequences for nutrients deficiency tolerance is not known. Understanding the physiological mechanisms may facilitate future breeding programs. One objective of the present work was to evaluate the functional significance of antioxidant defense capacity of plants in adaptation to Zn and Fe deficiency in combination with flooded conditions. Two contrasting rice genotypes used in this work, differed in tolerance to Zn and Fe deficiency and hypoxia. An attempt was also made to determine that up to what extent photosynthesis and related characteristics are associated with the tolerance to Zn and Fe deficiency and hypoxia.

Materials and Methods

Two genotypes of rice (*Oryza sativa* L.) were used in this work, one genotype (Amol) was chosen from north of Iran and another (Gasrol-Dashti) from south of the country because of their adaptation to their own local culture conditions. Seeds were provided by Rice Research Center (Rasht, Guilan Province, Iran) and Agricultural Research Center (Shiraz, Fars Province, Iran) for Amol and Dashti genotypes respectively.

Plants culture and treatments

The experiments were conducted in a growth chamber with a temperature regime of 25°/18°C day/night, 14/10 h light/dark period and relative humidity of 70/80% under photon flux density of 350-400 μmol m^{-2} s^{-1}. Germination of seeds and plants pre-culture were performed as described previously (Hajiboland and Salehi 2006a).

Zinc experiments

Conventional nutrient solution (Yoshida et al. 1972) was used for pre-culture of plants with Zn concentration of 0.5 μM (adequate Zn, control) and <0.08 μM (low). In the following growth period, it was necessary to use chelator-buffered nutrient solution technique (Yang et al. 1994b) for elimination of Zn contaminations and production of Zn deficient plants. Thus, sixteen-day-old plants were transferred to chelator-buffered nutrient solution consisted of two levels of added $ZnSO_4$ at 2.0 μM which equals 12 pM free Zn^{2+} activity (–Zn) or 20 μM equals 130 pM free Zn^{2+} activity (+Zn).

Fe experiments

Plants were grown in the conventional rice nutrient solution either in pre-culture or main experiment. Fe concentration in the pre-culture medium was 100 μM (adequate Fe) or 10 μM (low) and in the main experiment was 100 μM (adequate Fe, +Fe) or zero (–Fe).

Plants were grown for 21 days and nutrient solutions were completely changed every 7 days and pH was adjusted every day.

Hypoxia treatments

For comparison of growth of plants under hypoxic and aerobic conditions, plants were cultivated simultaneous in aerated

and non-aerated nutrient solutions during pre-culture as well as treatment.

Nutrient solutions were completely changed every 7 days and pH was adjusted every day.

Harvest

After washing using double-distilled water, plants were divided into shoots and roots, weighed and blotted dry on filter paper and dried at 70°C for 2 days to determine plant dry weight. Another group of plants was used for determination of root length (Tennant 1975) and chlorophyll (Moran 1982). The third group of plants was used for measurement of gas exchange parameters, chlorophyll fluorescence and determination of enzymes activity and concentration of metabolites.

Determination of gas exchange parameters

CO_2 assimilation and transpiration rates of attached leaves were measured with a calibrated portable gas exchange system (LCA-4, ADC Bioscientific Ltd., UK) always between 9:00 A.M. and 13:00 P.M. with the exception of PPFD (400-420 μmol m^{-2} s^{-1}), no microenvironmental variable inside the chamber was controlled. The net photosynthesis rate by unit of leaf area (*A*), transpiration rate (*E*), stomatal conductance to water vapor (g_s), atmospheric CO_2 molar fraction (*Ci/Ca*) and photosynthetic water use efficiency (*WUE=A/E*) were determined

Determination of chlorophyll fluorescence

Chlorophyll fluorescence parameters were recorded in parallel for gas exchange measurements in the same leaf, using a portable fluorometer (FIM, ADC Bioscientific Ltd., UK). Leaves were acclimated to dark for 30 min before measurements were taken. Initial (F_0), maximum (F_m), variable ($F_v=F_m-F_0$) as well as $F_v:F_m$ and $F_v:F_0$ ratios were recorded.

Gas exchange and chlorophyll fluorescence measurements were carried out using four independent replications as separate pots, in each pot four mature, fully expanded and attached leaves used for measurements. Average of data from each pot was obtained and the mean of four pots per treatment were subjected to statistical analysis.

Determination of enzyme activities and concentration of oxidants, total amino acids and protein

Fresh leaf samples were ground in the presence of liquid nitrogen using mortar and pestle. Each enzyme assay was tested for linearity between the volume of crude extract and the measured activity. All measurements were undertaken using spectrophotometer (Specord 200, Analytical Jena, Germany) according to optimized protocols described elsewhere (Hajiboland and Hasani 2007).

The activity of ascorbate peroxidase (APX, EC 1.11.1.11) was measured by determining ascorbic acid oxidation, one unit of APX oxidizes ascorbic acid at a rate of 1 μmol min^{-1} at 25°C. Catalase (CAT, EC 1.11.1.6) activity was assayed by monitoring the decrease in absorbance of H_2O_2 at 240 nm, unit activity was taken as the amount of enzyme, which decomposes 1 μmol of H_2O_2 in one min. Peroxidase (POD, EC 1.11.1.7) activity was assayed using the guaiacol test, the enzyme unit was calculated as enzyme protein required for the formation of 1 μmol tetraguaiacol for 1 min. Total superoxide dismutase (SOD, EC 1.15.1.1) activity was determined using monoformazan formation test. One unit of SOD was defined as the amount of enzyme required to induce a 50% inhibition of NBT reduction as measured at 560 nm, compared with control samples without enzyme aliquot. The activity of glutathione reductase (GR, EC 1.6.4.2) was assayed by following the oxidation of NADPH at 340 nm, one unit of enzyme activity was calculated as enzyme protein required for oxidation of one μmol NADPH in 1 min.

Soluble proteins were determined using a commercial Bradford reagent (Sigma) and BSA (Merck) as standard. Content of total free α-amino acids was assayed using ninhydrin colorimetric method at 570 nm, glycine was used for production of standard curve. The concentration of H_2O_2 was determined using potassium titanium-oxalate at 508 nm. Lipid peroxidation was estimated from the amount of malondialdehyde (MDA) formed in a reaction mixture containing thiobarbituric acid (Sigma) at 532 nm. MDA levels were calculated from a 1,1,3,3-tetraethoxypropane (Sigma) standard curve. The assay of NADPH-dependent $O_2^{\cdot-}$ generation was carried out by measuring the rate of SOD-inhibitable NBT reduction at 25°C.

Experiments were undertaken in complete randomized block design with 4 replications. Statistical analyses including one-way ANOVA (Tukey test) and Pearson Correlation Test were carried out using Sigma Stat (3.02) at $p<0.05$.

Results

Zinc deficiency caused significant reduction of shoot and root growth, however, genotypes differed markedly in tolerance to low Zn supply. Shoot growth of Amol was reduced by about 38%, while that of Dashti was inhibited up to 67%. Similar differential response of genotypes to low Zn stress was observed in root dry weight and length (Fig. 1).

In contrast to a high tolerance to low Zn supply, Amol was much more susceptible to Fe deficiency than Dashti. Growth reduction of Amol due to Fe deficiency was 69% and 67% for shoot of aerated and non-aerated plants respectively. The corresponding values for Dashti were only 52% and 55% under aerated and non-aerated conditions (Fig. 2).

Hypoxic conditions influenced plants growth depending on tested genotypes. In Zn and Fe sufficient plants, growth of Amol was stimulated up to 26% and 53% when grown

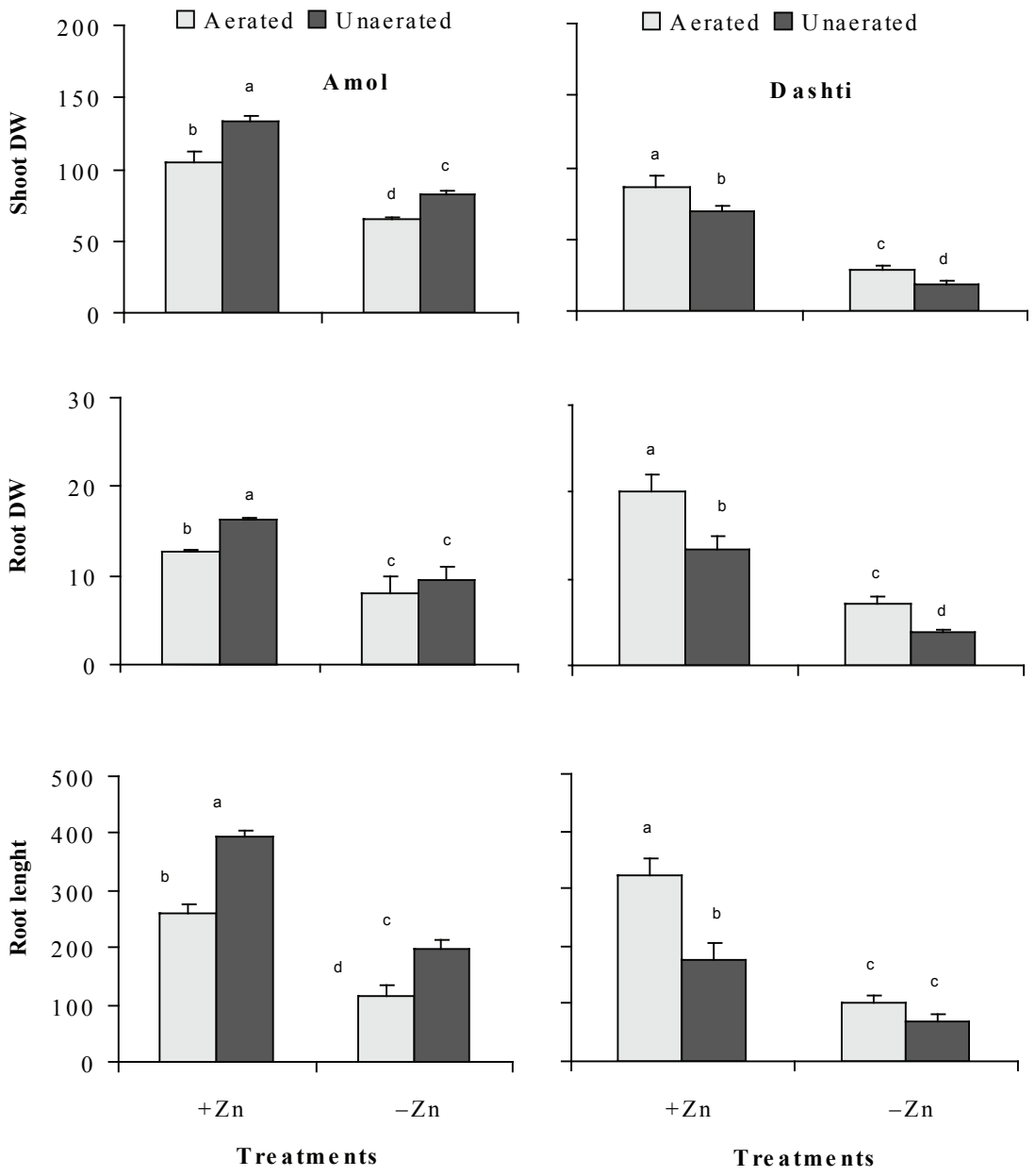

Figure 1. Effect of Zn deficiency on shoot and root dry weight (mg plant^{-1}) and root length (cm plant^{-1}) of two contrasting rice (*Oryza sativa* L. cvs. Amol and Dashti) genotypes grown under aerated or non-aerated conditions. Each value is the mean of 4 repetitions ± SD.

in non-aerated nutrient solution compared with aeration treatment in Zn and Fe experiment respectively. In contrast, Dashti showed higher root and shoot growth in aerated treatment, shoot biomass reduction due to growth in non-aerated nutrient solution was 21% and 33% in Zn and Fe experiment respectively. The same tendency was observed for Zn and Fe deficient plants (Figs. 1, 2).

Zinc deficiency did not affect the sum of chlorophyll a and b content (chlorophyll a+b) in Amol, but decreased it in Dashti particularly in non-aerated plants. Hypoxic conditions caused a slight increase of chlorophyll a+b amounts in both genotypes. F_v/F_m and F_v/F_0 ratios were not affected either by Zn deficiency or aeration treatments (Table 1).

Zinc deficient plants showed significantly lower net photosynthesis rate (*A*). Reduction of *A* was higher in Dashti (65-74%) than in Amol (47-54%). Hypoxic conditions did not affect *A* in both tested genotypes. Reduction of *A* was associated by great reduction of stomatal conductance (g_s). In addition of net photosynthesis rate, transpiration (*E*) was also affected negatively by low Zn supply, reduction of *E* by

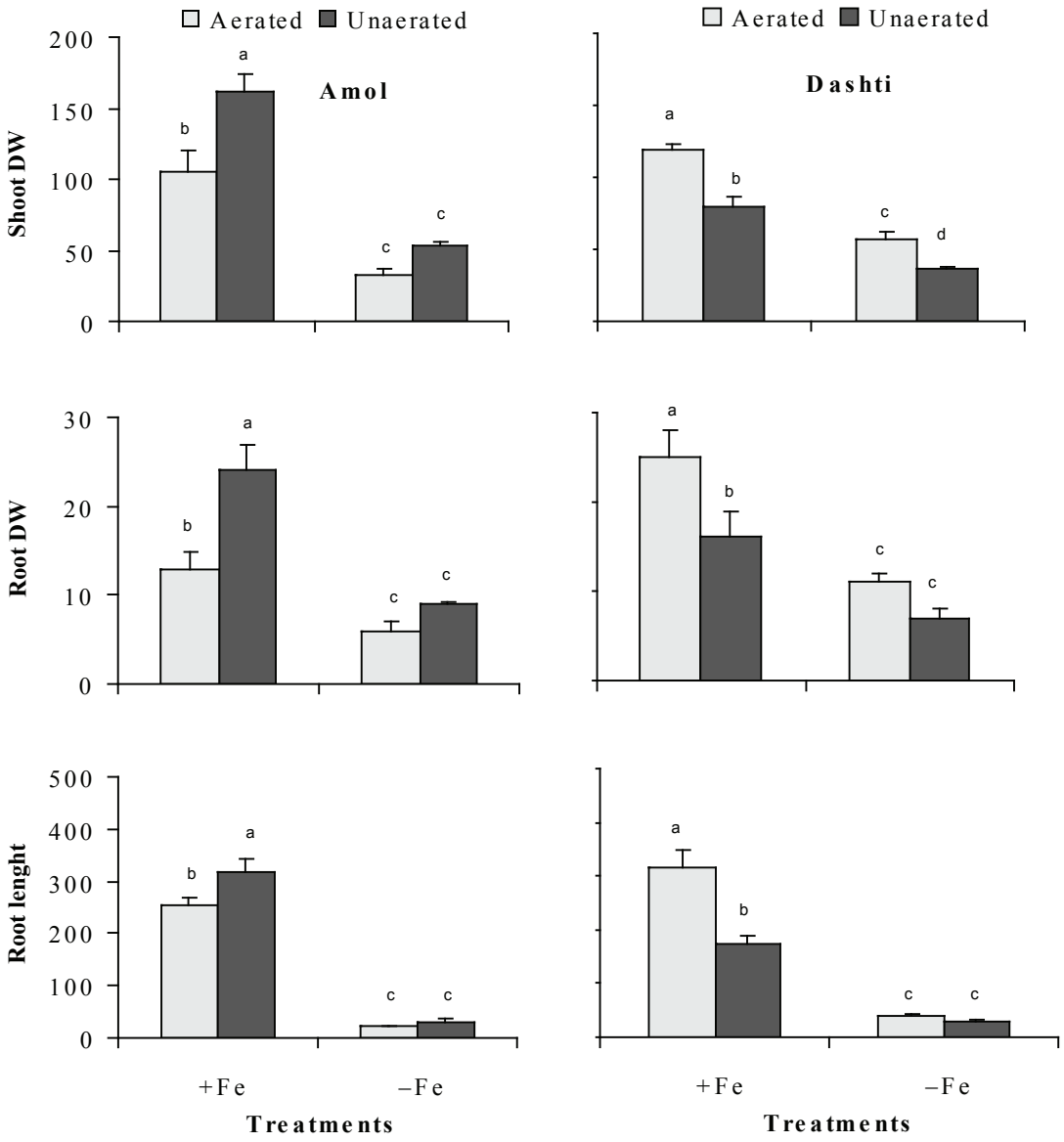

Figure 2. Effect of Fe deficiency on shoot and root dry weight (mg plant^{-1}) and root length (cm plant^{-1}) of two contrasting rice (*Oryza sativa* L. cvs. Amol and Dashti) genotypes grown under aerated or non-aerated conditions. Each value is the mean of 4 repetitions ± SD.

low Zn was higher in Dashti (67-69%) than Amol (43-52%). As the consequence of lower stomatal conductance, the *Ci/Ca* ratio increased in Zn deficient plants. Water use efficiency (*WUE*) was diminished by both low Zn supply and aeration of nutrient solution (Table 1).

As expected, the amount of chlorophyll a+b in leaves decreased dramatically in Fe deficient plants and in contrast to the effect of Zn deficiency, F_v/F_m ratio was significantly reduced by low supply of Fe. Reduction of optimal quantum efficiency of PSII in dark-adapted chloroplasts (F_v/F_m ratio) due to Fe deficiency was greater in Amol (34-17%) than Dashti (24-8%). Moreover, in Fe deficient plants, the F_v/F_m ratio was significantly affected by hypoxic conditions e.g. decreased up to 21% and 18% in Amol and Dashti respectively. The ratio of F_v/F_0 decreased with low Fe supply in both tested genotypes. The reduction was more pronounced in aerated (62-61% in Amol and Dashti respectively) than non-aerated (46-30% in Amol and Dashti respectively) plants (Table 2).

Similar with Zn deficiency, Fe deficiency conditions affected also negatively net photosynthesis rate, reduction was greater in Amol (74-81%) than Dashti (65-70%). Transpiration rate was also reduced by Fe deficiency, more in Amol (53%) than Dashti (26-33%). In accordance with the data of net photosynthesis and transpiration rate, stomatal con-

Table 1. Chlorophyll (a+b) concentration (mg g^{-1} FW), chlorophyll fluorescence and gas exchange parameters including net photosynthetic rate (*A*), transpiration (*E*), the ratio of intercellular air space and atmospheric CO_2 molar fractions (*Ci/Ca*), stomatal conductance to water vapor (*gs*) and instantaneous water use efficiency (*WUE*) in two contrasting rice genotypes (*Oryza sativa* L. cvs. Amol and Dashti) treated either with adequate or low levels of Zn under aerated or non-aerated conditions. The means refer to 4 repetitions ± SD. Data within each genotype followed by the same letter are not significantly different (P<0.05).

		Amol		Dashti	
		Aerated	Non-aerated	Aerated	Non-aerated
Chlorophyll (a+b)	+Zn	2.96±0.62 ^{a}	3.22±0.48 ^{a}	2.77±0.29 ^{ab}	3.16±0.59 ^{a}
	–Zn	1.81±0.09 ^{b}	1.93±0.16 ^{b}	2.33±0.49 ^{ab}	1.97±0.23 ^{b}
Fv/Fm	+Zn	0.788±0.002 ^{a}	0.794±0.005 ^{a}	0.800±0.004 ^{a}	0.802±0.004 ^{a}
	–Zn	0.792±0.006 ^{a}	0.797±0.001 ^{a}	0.806±0.010 ^{a}	0.807±0.004 ^{a}
Fv/F_0	+Zn	3.73±0.04 ^{b}	3.86±0.11 ^{b}	4.02±0.11 ^{a}	4.13±0.24 ^{a}
	–Zn	4.84±0.12 ^{a}	3.92±0.03 ^{b}	4.17±0.28 ^{a}	4.20±0.11 ^{a}
A ($\mu mol\ m^{-2}\ s^{-1}$)	+Zn	1.12±0.14 ^{a}	1.31±0.10 ^{a}	1.58±0.11 ^{a}	1.50±0.10 ^{a}
	–Zn	0.59±0.10 ^{c}	0.60±0.06 ^{b}	0.56±0.09 ^{b}	0.39±0.09 ^{b}
E ($mmol\ m^{-2}\ s^{-1}$)	+Zn	0.64±0.18 ^{a}	0.48±0.06 ^{abc}	0.83±0.06 ^{a}	0.73±0.12 ^{a}
	–Zn	0.36±0.13 ^{bc}	0.23±0.02 ^{c}	0.26±0.04 ^{b}	0.24±0.07 ^{b}
g_s ($mol\ m^{-2}\ s^{-1}$)	+Zn	12.67±5.03 ^{a}	8.67±4.20 ^{a}	17.33±3.05 ^{a}	15.33±4.16 ^{a}
	–Zn	2.00±0.46 ^{b}	1.01±0.01 ^{c}	4.02±0.01 ^{b}	2.00±0.02 ^{c}
Ci/Ca	+Zn	0.548±0.034 ^{b}	0.484±0.024 ^{c}	0.493±0.033 ^{b}	0.489±0.031 ^{b}
	–Zn	0.705±0.029 ^{a}	0.648±0.027 ^{a}	0.689±0.022 ^{a}	0.737±0.039 ^{a}
WUE ($\mu mol\ mol^{-1}$)	+Zn	1.81±0.34 ^{b}	2.78±0.27 ^{a}	1.92±0.27 ^{ab}	2.08±0.23 ^{a}
	–Zn	1.63±0.40 ^{b}	2.66±0.18 ^{a}	2.12±0.17 ^{a}	1.59±0.06 ^{b}

Table 2. Chlorophyll (a+b) concentration (mg g^{-1} FW), chlorophyll fluorescence and gas exchange parameters including net photosynthetic rate (*A*), transpiration (*E*), the ratio of intercellular air space and atmospheric CO_2 molar fractions (*Ci/Ca*), stomatal conductance to water vapor (*gs*) and instantaneous water use efficiency (*WUE*) in two contrasting rice genotypes (*Oryza sativa* L. cvs. Amol and Dashti) treated either with adequate or low levels of Fe under aerated or non-aerated conditions. The means refer to 4 repetitions ± SD. Data within each genotype followed by the same letter are not significantly different (P<0.05).

		Amol		Dashti	
		Aerated	Non-aerated	Aerated	Non-aerated
Chlorophyll (a+b)	+Fe	1.84±0.01 ^{b}	3.03±0.05 ^{a}	1.97±0.13 ^{b}	2.84±0.53 ^{a}
	–Fe	0.07±0.00 ^{d}	0.25±0.07 ^{c}	0.15±0.05 ^{c}	0.39±0.17 ^{c}
Fv/Fm	+Fe	0.785±0.004 ^{a}	0.791±0.001 ^{a}	0.801±0.006 ^{a}	0.805±0.003 ^{ab}
	–Fe	0.518±0.010 ^{c}	0.653±0.011 ^{b}	0.608±0.042 ^{c}	0.739±0.040 ^{b}
Fv/F_0	+Fe	3.64±0.06 ^{b}	3.80±0.02 ^{a}	3.94±0.26 ^{a}	4.15±0.07 ^{a}
	–Fe	1.38±0.07 ^{d}	2.04±0.14 ^{c}	1.52±0.20 ^{c}	2.91±0.18 ^{b}
A ($\mu mol\ m^{-2}\ s^{-1}$)	+Fe	0.93±0.20 ^{a}	0.94±0.07 ^{a}	1.19±0.26 ^{a}	0.89±0.24 ^{a}
	–Fe	0.18±0.03 ^{b}	0.24±0.08 ^{b}	0.34±0.13 ^{b}	0.31±0.05 ^{b}
E ($mmol\ m^{-2}\ s^{-1}$)	+Fe	0.51±0.09 ^{a}	0.40±0.06 ^{a}	0.61±0.10 ^{a}	0.63±0.14 ^{a}
	–Fe	0.24±0.11 ^{b}	0.19±0.01 ^{b}	0.45±0.13 ^{a}	0.42±0.19 ^{a}
g_s ($mol\ m^{-2}\ s^{-1}$)	+Fe	6.67±3.05 ^{a}	2.78±0.55 ^{b}	10.00±4.00 ^{a}	8.67±2.43 ^{a}
	–Fe	1.00±0.01 ^{c}	1.08±0.00 ^{c}	5.33±1.16 ^{ab}	3.00±0.76 ^{b}
Ci/Ca	+Fe	0.557±0.016 ^{c}	0.622±0.018 ^{b}	0.463±0.013 ^{c}	0.504±0.014 ^{b}
	–Fe	1.089±0.031 ^{a}	1.046±0.028 ^{a}	0.836±0.023 ^{a}	0.828±0.023 ^{a}
WUE ($\mu mol\ mol^{-1}$)	+Fe	1.82±0.11 ^{ab}	2.39±0.35 ^{a}	2.15±0.59 ^{a}	1.48±0.64 ^{ab}
	–Fe	0.89±0.54 ^{c}	1.26±0.44 ^{bc}	0.62±0.10 ^{c}	0.73±0.12 ^{bc}

ductance was strongly inhibited by Fe deficiency, and more reduction was observed in Amol (85-97%) compared with Dashti (48-65%). Water use efficiency (*WUE*) decreased in Fe deficient plants with no obvious genotypic difference, moreover, a slight reduction of *WUE* was observed in aerated plants (Table 2).

A significant increase in the specific activity of APX was observed in roots of non-aerated plants by low Zn supply that reached up to 46% and 65% in Amol and Dashti respectively. This increase in shoot was in tendency and observed only in Amol. On the other hand, APX activity increased in Zn deficient Amol plants by hypoxic treatment, which was significant

Table 3. Effect of Zn deficiency on the specific activity of ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and glutathione reductase (GR) in two genotypes of rice (*Oryza sative* L. cvs. Amol and Dashti) grown in aerated or non-aerated nutrient solution. Data in each column within each genotype followed by the same letter are not significantly different ($P<0.05$).

Genotypes	Treatments		APX nmol H_2O_2 mg^{-1} protein min^{-1}	CAT µmol H_2O_2 mg^{-1} protein min^{-1}	POD µmol Guaiacol mg^{-1} protein min^{-1}	SOD Unit mg^{-1} protein	GR nmol NADPH mg^{-1} protein min^{-1}
			Shoot				
Amol	Aer.	+Zn	42.6±15.5 [a]	5.04±0.62 [a]	0.13±0.01 [b]	7.57±0.72 [a]	12.2±0.6 [a]
		–Zn	51.5±1.8 [a]	5.82±0.52 [a]	0.18±0.02 [a]	4.91±0.61 [b]	10.8±2.6 [a]
	Unaer.	+Zn	45.3±9.9 [a]	6.66±0.72 [a]	0.13±0.02 [b]	8.89±0.96 [a]	9.7±0.8 [a]
		–Zn	67.8±8.7 [a]	5.49±0.64 [a]	0.19±0.01 [a]	5.54±0.23 [b]	10.4±1.7 [a]
Dashti	Aer.	+Zn	52.1±9.3 [a]	4.63±0.37 [a]	0.14±0.01 [b]	8.35±0.68 [a]	8.9±1.8 [b]
		–Zn	47.2±4.7 [a]	4.85±0.78 [a]	0.18±0.02 [b]	3.94±1.00 [b]	7.9±1.5 [b]
	Unaer.	+Zn	49.2±8.9 [a]	4.72±0.40 [a]	0.15±0.02 [b]	7.96±0.84 [a]	12.2±0.8 [a]
		–Zn	40.1±5.9 [a]	3.86±0.52 [a]	0.39±0.03 [a]	3.16±0.53 [b]	12.6±0.7 [a]
			Root				
Amol	Aer.	+Zn	46.3±0.8 [b]	2.64±0.51 [a]	1.02±0.09 [b]	7.89±0.81 [a]	10.7±3.9 [a]
		–Zn	58.2±2.6 [b]	3.97±0.84 [a]	1.73±0.08 [a]	5.34±0.42 [b]	9.8±3.9 [a]
	Unaer.	+Zn	53.3±0.8 [b]	2.37±0.18 [a]	1.03±0.15 [b]	8.77±0.95 [a]	11.9±0.7 [a]
		–Zn	88.2±10.4 [a]	3.23±0.84 [a]	1.80±0.25 [a]	5.69±0.41 [b]	10.9±1.4 [a]
Dashti	Aer.	+Zn	26.0±3.9 [b]	1.25±0.06 [a]	0.60±0.02 [b]	8.49±1.17 [a]	8.6±0.5 [a]
		–Zn	34.9±2.3 [ab]	1.20±0.06 [a]	0.54±0.01 [c]	6.56±0.55 [b]	9.1±1.4 [a]
	Unaer.	+Zn	27.9±1.4 [b]	1.42±0.17 [a]	0.64±0.04 [b]	8.48±0.67 [a]	10.2±1.4 [a]
		–Zn	40.7±9.2 [a]	1.30±0.13 [a]	1.16±0.04 [a]	5.82±0.37 [b]	9.2±1.6 [a]

in roots (52%) but in tendency in shoot. Activity of CAT did not respond either to low Zn stress or aeration treatment. In contrast, activity of POD responded to both Zn and aeration treatments in shoot and root. Zn deficiency induced activity of POD (with the exception of aerated Dashti) up to 46% and 160% for shoot and 75% and 81% for roots of non-aerated Amol and Dashti, respectively. As expected, Zn deficiency caused reduction of SOD activity in both shoot and root irrespective to aeration treatment. The most obvious response was detected in Dashti with up to 53% and 60% reduction of SOD activity under aerated and non-aerated conditions, respectively. Activity of GR did not respond to low Zn supply and hypoxia, with the exception of Dashti, in which aeration caused significant reduction of GR activity up to 48% in shoot (Table 3).

A consistent, though non significant reduction of APX activity was observed due to Fe starvation in both genotypes. As expected, activity of CAT decreased strongly by low Fe supply; considering relative amounts of reduction (% over control), inhibition of CAT activity was mainly higher in Amol than Dashti and in aerated than non-aerated plants. Similar with CAT, POD activity was also inhibited by low Fe supply. In contrast, activity of SOD increased in shoot and roots of Fe deficient plants compared with control, this effect was more prominent in Amol than Dashti. Activity of GR increased in Fe deficient plants, which was mainly in tendency and non significant (Table 4).

Concentration of H_2O_2 responded to Zn deficiency in shoot and root. In shoot, Zn deficiency caused a reduction in concentration of H_2O_2, but in roots an increase of H_2O_2 concentration was observed. Aeration caused higher accumulation of H_2O_2, significantly or in tendency. Superoxide radicals ($O_2^{\cdot-}$) accumulated in Zn deficient plants in both genotypes, however, aeration treatments had different effect on $O_2^{\cdot-}$ concentration depending on genotype. In Amol, aeration caused an increase of $O_2^{\cdot-}$ concentration in both Fe sufficient and deficient plants, in contrast, in Dashti an accumulation of $O_2^{\cdot-}$ was observed in plants grown under hypoxic conditions. Concentration of MDA increased in response to Zn deficiency. Similar with $O_2^{\cdot-}$, aeration showed a differential effect on MDA concentration, e.g. reduction in Amol and increase in Dashti under hypoxia (Table 5).

Concentration of H_2O_2 increased by low Fe supply in root, however, the opposite was observed for shoot, its concentration was lower in Fe deficient compared with control plants. Concentration of $O_2^{\cdot-}$ increased by low Fe supply, moreover, MDA concentration increased significantly or in tendency in Fe deficient plants (Table 6).

Zinc deficiency caused reduction of protein concentration in both shoot and root and in both genotypes. In this respect, two tested genotypes did not differ in their response to Zn deficiency. In accordance with the results of protein concentration, free amino acids accumulated significantly or in tendency in both shoot and root of low Zn plants (Table 5).

Table 4. Effect of Fe deficiency on the specific activity of ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and glutathione reductase (GR) in two genotypes of rice (*Oryza sative* L. cvs. Amol and Dashti) grown in aerated or non-aerated nutrient solution. Data in each column within each genotype followed by the same letter are not significantly different ($P<0.05$).

Genotypes	Treatments		APX nmol H_2O_2 mg^{-1} protein min^{-1}	CAT µmol H_2O_2 mg^{-1} protein min^{-1}	POD µmol Guaiacol mg^{-1} protein min^{-1}	SOD Unit mg^{-1} protein	GR nmol NADPH mg^{-1} protein min^{-1}
			Shoot				
Amol	Aer.	+Fe	46.5±17.3 ^{a}	4.66±0.14 ^{b}	0.11±0.01 ^{b}	9.79±0.85 ^{b}	14.4±2.9 ^{a}
		–Fe	40.9±6.9 ^{ab}	1.16±0.06 ^{c}	0.08±0.00 ^{c}	13.26±1.00 ^{a}	15.2±0.6 ^{a}
	Unaer.	+Fe	48.1±7.1 ^{a}	5.34±0.43 ^{a}	0.14±0.01 ^{a}	9.96±0.46 ^{b}	11.2±0.7 ^{a}
		–Fe	23.6±2.9 ^{b}	1.39±0.18 ^{c}	0.09±0.00 ^{c}	13.44±1.85 ^{a}	13.2±0.9 ^{a}
Dashti	Aer.	+Fe	46.4±2.5 ^{a}	4.12±0.67 ^{a}	0.17±0.02 ^{ab}	9.42±0.99 ^{ab}	9.2±0.9 ^{b}
		–Fe	30.5±8.9 ^{ab}	1.20±0.20 ^{b}	0.12±0.01 ^{c}	10.89±0.67 ^{a}	10.1±1.5 ^{ab}
	Unaer.	+Fe	39.2±12.4 ^{ab}	4.22±0.48 ^{a}	0.19±0.02 ^{a}	8.87±1.02 ^{b}	10.9±0.4 ^{ab}
		–Fe	21.6±1.7 ^{b}	1.48±0.05 ^{b}	0.15±0.01 ^{b}	10.47±1.00 ^{ab}	12.3±0.6 ^{a}
			Root				
Amol	Aer.	+Fe	46.9±1.3 ^{b}	1.57±0.27 ^{a}	1.02±0.04 ^{a}	7.11±1.11 ^{b}	10.9±0.7 ^{b}
		–Fe	45.3±5.7 ^{b}	0.36±0.05 ^{c}	0.72±0.09 ^{b}	12.71±1.21 ^{a}	13.7±0.9 ^{a}
	Unaer.	+Fe	60.2±7.4 ^{a}	0.82±0.09 ^{b}	1.07±0.16 ^{a}	7.38±0.87 ^{b}	14.3±1.1 ^{a}
		–Fe	52.7±3.8 ^{ab}	0.38±0.05 ^{c}	0.81±0.03 ^{b}	13.68±1.58 ^{a}	14.4±2.2 ^{ab}
Dashti	Aer.	+Fe	30.9±3.9 ^{a}	1.01±0.05 ^{b}	0.44±0.01 ^{a}	10.11±0.46 ^{b}	10.1±0.8 ^{a}
		–Fe	25.4±7.1 ^{a}	0.22±0.02 ^{d}	0.31±0.01 ^{b}	12.88±1.03 ^{a}	12.4±3.5 ^{a}
	Unaer.	+Fe	42.3±12.1 ^{a}	1.23±0.11 ^{a}	0.47±0.03 ^{a}	9.63±1.24 ^{b}	12.0±1.6 ^{a}
		–Fe	35.3±3.9 ^{a}	0.68±0.09 ^{c}	0.35±0.05 ^{b}	11.78±1.00 ^{ab}	13.5±0.1 ^{a}

Similar with Zn, Fe deficiency caused reduction of protein concentration and increase in total amino acids concentration, the latter change was mainly in tendency (Table 6).

Discussion

A distinct genotypic difference was observed in plants response to hypoxic conditions. Dry matter production of the lowland genotype (Amol) was significantly improved in nutrient solution without aeration, the opposite was observed for flooding sensitive genotype (Dashti). Root length was also affected by aeration treatments differently. Differential response of root length to hypoxia may have important consequences for nutrients acquisition of plants grown in soils with different oxygen availability.

Zinc deficiency tolerance of Amol was greater than Dashti. Amol has been characterized as an extremely Zn-efficient genotype in our previous work (Hajiboland and Salehi 2006a). Our experiment demonstrated also that, there is no direct relationship between tolerance to flooding and Zn deficiency in Iranian rice genotypes (Hajiboland and Salehi 2006b). In contrast, susceptibility to Fe deficiency was obviously related to flooding response in tested genotypes in this work as judged by biomass and chlorophyll content. Co-occurrence of flooding and high available Fe in soils is likely the cause of this relationship. Flooding causes the soil to become anaerobic with a low redox potential leading to a high and probably near toxic Fe^{2+} availability. Therefore, lowland rice genotypes have developed in an edaphic environment, in which there is no need for absorbing Fe with high efficiency that in turn has been led to higher susceptibility to Fe deficiency. In contrast, drained soils particularly calcareous ones are deficient in available Fe. Accordingly, Fe deficiency tolerance has been developed during selection and improvement of upland genotype.

Zinc deficiency did not affect strongly photochemistry of leaves, suggesting that though production of more active oxygen species in Zn deficient plants in this work, thylakoid constituents has not been damaged seriously. It was suggested that, for nutrients without direct involvement in the electron transport or chlorophyll synthesis such as Zn, a close linkage between nutritional status of leaves and spectral characteristics seems unlikely (Adams et al. 2000). However, there are reports on significant reduction of maximum quantum efficiency of PSII (Wang and Jin 2005) and severe damage to the ultrastructure of chloroplasts (Chen et al. 2007) in plants subjected to inadequate Zn supply. It is likely that, the severity and/or duration of Zn deficiency stress in our experiment were not enough for induction of serious damage to photosynthetic membranes and disturbance in the photochemistry of leaves.

In contrast, Fe deficiency depressed strongly maximal quantum efficiency of PSII (F_v/F_m). Reduction in F_v/F_m was due to both decrease in the electron transport chain (reduction of F_m) and particularly due to structural modifications of PSII mainly at the pigment level (increase in F_0). The negative effect of Fe deficiency for both rice genotypes focused mainly

Table 5. Effect of Zn deficiency on the concentration of protein (mg g^{-1} FW), total free a-amino acids (TAA), hydrogen peroxide (H_2O_2), superoxide radicals ($O_2^{\cdot-}$) and malondialdehyde (MDA) in two genotypes of rice (*Oryza sative* L. cvs. Amol and Dashti) grown in aerated or non-aerated nutrient solution. Data in each column within each genotype followed by the same letter are not significantly different (P<0.05).

			H_2O_2	$O_2^{\cdot-}$	MDA	Protein	TAA
Genotypes	Treatments		nmol g^{-1} FW	nmol g^{-1} FW	µmol g^{-1} FW	mg g^{-1} FW	mmol g^{-1} FW
			Shoot				
	Aer.	+Zn	1305±176 ^{a}	nd	1.49±0.29 ^{b}	59.5±2.1 ^{a}	262±33 ^{c}
Amol		–Zn	934±211 ^{a}	nd	2.74±0.69 ^{a}	42.7±2.7 ^{b}	369±23 ^{a}
	Unaer.	+Zn	1289±56 ^{a}	nd	1.05±0.91 ^{b}	60.2±2.3 ^{a}	208±20 ^{d}
		–Zn	1167±110 ^{a}	nd	1.32±0.31 ^{ab}	45.9±3.2 ^{b}	312±11 ^{b}
	Aer.	+Zn	1748±166 ^{a}	nd	3.24±0.98 ^{c}	65.8±4.2 ^{a}	183±82 ^{b}
Dashti		–Zn	669±36 ^{c}	nd	5.01±0.33 ^{ab}	52.7±1.6 ^{b}	289±31 ^{ab}
	Unaer.	+Zn	1077±38 ^{b}	nd	4.34±0.43 ^{bc}	68.1±1.5 ^{a}	276±3 ^{a}
		–Zn	843±116 ^{c}	nd	6.01±0.71 ^{a}	55.9±1.8 ^{b}	361±29 ^{a}
			Root				
	Aer.	+Zn	20.8±8.1 ^{b}	149±23 ^{c}	0.98±0.15 ^{a}	17.6±1.5 ^{b}	25.3±2.4 ^{b}
Amol		–Zn	40.5±12.7 ^{a}	259±30 ^{a}	1.04±0.15 ^{a}	13.3±1.5 ^{c}	38.3±1.9 ^{a}
	Unaer.	+Zn	6.5±1.7 ^{b}	108±19 ^{d}	0.52±0.09 ^{b}	21.3±1.4 ^{a}	27.4±2.2 ^{b}
		–Zn	18.5±5.4 ^{b}	191±8 ^{b}	0.83±0.19 ^{a}	17.1±1.5 ^{b}	36.2±2.7 ^{a}
	Aer.	+Zn	18.8±2.3 ^{c}	185±21 ^{c}	2.17±0.17 ^{b}	30.7±3.2 ^{a}	28.9±4.3 ^{b}
Dashti		–Zn	30.0±4.8 ^{b}	415±38 ^{b}	3.41±0.84 ^{ab}	24.7±0.7 ^{b}	37.3±1.3 ^{a}
	Unaer.	+Zn	17.6±3.8 ^{c}	187±29 ^{c}	2.54±0.26 ^{ab}	29.2±2.4 ^{a}	35.8±3.1 ^{ab}
		–Zn	42.2±8.1 ^{a}	531±82 ^{a}	3.95±0.89 ^{a}	23.8±2.3 ^{b}	31.3±2.5 ^{ab}

Table 6. Effect of Fe deficiency on the concentration of protein (mg g^{-1} FW), total free a-amino acids (TAA), hydrogen peroxide (H_2O_2), superoxide radicals ($O_2^{\cdot-}$) and malondialdehyde (MDA) in two genotypes of rice (*Oryza sative* L. cvs. Amol and Dashti) grown in aerated or non-aerated nutrient solution. Data in each column within each genotype followed by the same letter are not significantly different (P<0.05).

			H_2O_2	$O_2^{\cdot-}$	MDA	Protein	TAA
Genotypes	Treatments		nmol g^{-1} FW	nmol g^{-1} FW	µmol g^{-1} FW	mg g^{-1} FW	mmol g^{-1} FW
			Shoot				
	Aer.	+Fe	1853±429 ^{a}	nd	1.22±0.30 ^{a}	58.5±1.6 ^{a}	293±17 ^{ab}
Amol		–Fe	424±19 ^{c}	nd	1.35±0.12 ^{a}	35.8±1.2 ^{c}	350±46 ^{a}
	Unaer.	+Fe	1347±74 ^{b}	nd	1.06±0.20 ^{a}	60.1±1.9 ^{a}	257±7 ^{b}
		–Fe	344±32 ^{c}	nd	1.34±0.28 ^{a}	40.1±0.9 ^{b}	284±42 ^{ab}
	Aer.	+Fe	1367±236 ^{a}	nd	4.41±0.08 ^{b}	59.8±4.2 ^{a}	241±23 ^{a}
Dashti		–Fe	367±38 ^{b}	nd	5.58±1.69 ^{b}	44.7±1.5 ^{b}	293±75 ^{a}
	Unaer.	+Fe	1254±159 ^{a}	nd	5.62±1.92 ^{b}	59.9±1.9 ^{a}	249±30 ^{a}
		–Fe	215±69 ^{b}	nd	10.23±1.79 ^{a}	45.7±1.6 ^{b}	294±15 ^{a}
			Root				
	Aer.	+Fe	45.9±4.8 ^{b}	233±20 ^{c}	2.13±0.98 ^{c}	20.5±1.2 ^{a}	28.1±1.5 ^{a}
Amol		–Fe	59.5±6.2 ^{a}	584±55 ^{a}	5.75±0.00 ^{a}	13.2±0.4 ^{b}	34.2±2.6 ^{a}
	Unaer.	+Fe	9.2±2.9 ^{c}	227±24 ^{c}	1.52±0.37 ^{c}	21.1±1.0 ^{a}	29.4±2.6 ^{a}
		–Fe	36.8±2.0 ^{b}	464±26 ^{b}	4.14±0.34 ^{b}	13.9±0.6 ^{b}	33.1±3.3 ^{a}
	Aer.	+Fe	21.9±3.8 ^{c}	201±17 ^{b}	2.80±0.20 ^{c}	31.7±2.5 ^{a}	27.4±2.8 ^{a}
Dashti		–Fe	40.3±4.1 ^{a}	359±25 ^{a}	12.40±1.47 ^{b}	24.8±0.6 ^{b}	32.9±3.9 ^{a}
	Unaer.	+Fe	19.5±0.5 ^{c}	222±23 ^{b}	3.98±1.07 ^{c}	29.6±1.6 ^{a}	28.7±1.8 ^{a}
		–Fe	32.2±3.3 ^{b}	361±13 ^{a}	19.09±4.85 ^{a}	24.4±1.4 ^{b}	28.9±1.1 ^{a}

on the decreased proportion of active chlorophyll associated with the reaction center of PSII (decreased F_v/F_0). Increase in F_0 could be originated from increases in the dark reduction of the plastoquinone pool (Belkhodja et al. 1998) or from the inactivation of the ferredoxin, an electron transmitter, due to changes in its chemical structure. Accordingly, in Fe deficient plants a close correlation was found between the rate of net photosynthesis and photochemical properties of leaves (Table 7), suggesting that inhibited light reactions in Fe deficient leaves contributes significantly in reduction of CO_2 assimila-

Table 7. Correlation coefficient between plants dry weight and net photosynthesis rate, photochemical properties of leaves, activity of antioxidant enzymes and concentration of oxidants in two genotypes of rice (*Oryza sative* L. cvs. Amol and Dashti) grown under different nutritional status of Zn and Fe. Shoot and root dry weight data and the values for activity of enzymes in these parts were subjected to analysis of their correlation. For analysis of correlation between photosynthetic parameters and plants DW, dry weight of shoot (but not root) was regarded. Data of two studied genotypes were combined. Coefficients above the dashed line refer to the Zn experiment and those below it to the Fe experiment. ns: non significant, * significant at 0.05, ** significant at 0.01.

	Plant DW	*A*	F_v/F_m	F_v/F_0	APX	CAT	POD	SOD	GR	H_2O_2	$O_2^{\cdot-}$	MDA
Plant DW	---	0.65 ns	-0.77 *	-0.49 ns	0.08 ns	0.81 **	-0.69 **	0.30 ns	0.12 ns	0.88 **	-0.79 **	-0.15 ns
A	0.85 **	---	-0.24 ns	-0.42 ns	-0.08 ns	0.16 ns	-0.70 ns	0.94 **	-0.06 ns	0.77 **	nd	-0.31 Ns
F_v/F_m	-0.71 *	0.86 **	---	---	-0.11 ns	-0.65 ns	0.59 ns	-0.49 ns	-0.18 ns	-0.41 ns	nd	0.86 **
F_v/F_0	0.73 **	0.90 **	---	---	0.02 ns	0.00 ns	0.25 ns	-0.50 ns	0.00 ns	-0.47 ns	nd	0.36 ns
APX	0.15 ns	0.76 **	0.38 ns	0.02 ns	---	---	---	---	---	0.11 ns	-0.21 ns	-0.39 ns
CAT	0.94 **	0.92 **	0.84 **	0.00 ns	---	---	---	---	---	0.81 **	0.39 ns	-0.04 ns
POD	-0.56 **	0.65 ns	0.76 **	0.25 ns	---	---	---	---	---	-0.76 **	-0.06 ns	-0.41 ns
SOD	0.26 ns	0.82 **	-0.85 **	-0.50 ns	---	---	---	---	---	0.01 ns	-0.60 ns	-0.51 *
GR	-0.36 ns	-0.48 ns	-0.46 ns	0.00 ns	---	---	---	---	---	0.15 ns	-0.60 ns	-0.08 ns
H_2O_2	0.90 **	0.91 **	0.74 ns	-0.47 ns	0.20 ns	0.95 **	-0.55 *	-0.24 ns	-0.19 ns	---	---	0.21 Ns
$O_2^{\cdot-}$	-0.79 *	nd	nd	nd	0.06 ns	-0.75 *	-0.04 ns	0.78 *	0.60 ns	---	---	0.85 **
MDA	-0.37 ns	-0.15 ns	0.15 ns	0.08 ns	-0.44 ns	-0.35 ns	-0.11 ns	0.26 ns	0.00 ns	0.32 ns	0.30 ns	---

tion. However, even in Fe deficient plants the contribution of stomatal closure in reduction of net assimilation rate (85%) was more pronounced than that of reduction in quantum efficiency of PSII (34%). In contrast, lower assimilation rate in low Zn plants was solely attributable to the stomatal limitation. Reduction of stomatal conductance due to low supply of Zn was reported for other plants such as chickpea (Rengel et al. 2004) and maize (Wang and Jin 2005). Stomatal limitation was also observed for Fe deficient plants (Chouliaras et al. 2004; Molassiotis et al. 2006).

Aeration of nutrient solution reduced stomatal conductance in tendency or significantly and in both tested genotypes. Therefore, plants grown under aerobic conditions lost more water via transpiration, leading to lower *WUE*. Change of water management and shift from flooded to aerobic conditions in rice fields was recommended because of increasing water scarcity in the world (Bouman et al. 2005). Our results, however, showed that rice plants would have use soil water resources more inefficiently when grown in drained soils compared with flooded fields. Decrease in stomatal conductance under flooded conditions has been demonstrated in many woody species of temperate and tropical forest ecosystems (Mielke et al. 2003) and was attributed to decrease in root hydraulic conductivity under soil anaerobic conditions leading to internal water stress and reducing leaf turgor (Pezeshki 2001) or production of abscisic acid (Zhang and Zhang 1994). Very limited information is available on the effect of flooding on stomatal behavior in leaves of soil grown rice plants (Ishihara and Saito 1987). However, a field study showed that the water productivity of rice under aerobic conditions was 32-88% higher than under flooded conditions (Bouman et al. 2005). In the calculation of water productivity the amount of evapotranspiration is considered (Kassam and Smith 2001), therefore, lower values for water productivity could be the result of higher water table evaporation in the flooded soils rather higher transpiration by plants. Nevertheless, our data for nutrient solution grown plants, in which flooded conditions was simulated by only one of several factors functioning in a submerged soils, should be considered only with great cautions.

Activity of two H_2O_2 scavenging enzymes, APX and POD was induced by low Zn supply. Induction of APX and particularly POD under the effect of deficiency of macro (Tewari et al. 2007) or micronutrients (Candan and Tarhan 2003) was frequently reported. In contrast to the effect of Zn, Fe caused a reduction of APX, CAT and POD. Iron is one component of prosthetic group of peroxidases, therefore reduction of activity of peroxidases in Fe deficient plants is

expected (Marschner 1995). Activity of CAT responded more strongly to Fe deficiency than APX and POD. In addition, a close correlation observed between activity of CAT, net CO_2 assimilation and maximum quantum efficiency of PSII in Fe deficient leaves (Table 7) further confirms the relevance of using its activity as an indicator of Fe nutritional status of plants (Marschner 1995).

Activity of GR did not seem to be responsive to Fe or Zn deficiency and GR seems to be not involved in antioxidant defense system of Zn and Fe starved plants. Glutathione reductase effect on protection of plants against stresses evoked by sulfur (Hajiboland and Amjad 2007), nitrogen and K (Tewari et al. 2007) deficiency was reported, likely because of its effect on keeping reduced GSH at a given level.

Greater APX activity in Amol under hypoxic conditions, which was associated with higher tolerance (rather a growth improvement) suggests possible role for this enzyme in protection of plants against hypoxia. Change in the activity of APX was reflected in H_2O_2 concentration in plants, *i.e.* higher APX activity in non-aerated Amol was associated with lower accumulation of H_2O_2 in plants; roots were more responsive than shoot. Induction of APX activity was suggested to provide plant roots with increased tolerance to waterlogged stress (Lin et al. 2004).

Activity of POD increased in Dashti treated with hypoxia, this treatment caused stress for plants as judged by lower growth. In addition, higher POD activity in non-aerated Dashti did not result in lower H_2O_2 concentration. As it is obvious from correlation coefficient presented in Table 7, there is rather a negative relationship between POD activity and plants growth, e.g. lower growth was accompanied by higher activity of POD. It implies that, POD activity only monitored stress conditions without any protecting role. The unspecific POD activity assayed with guaiacol as a universal substrate can exhibit activity of APX (antioxidant enzyme), coniferyl alcohol peroxidase (lignifying enzyme), NADH oxidase and IAA oxidase (growth limiting peroxidases). The individual activity of these enzymes with the exception of APX, were not distinguished from the soluble pool in our extraction procedure. On the other hand, the functional significance of peroxidases measured by guaiacol test in the protection of plants against oxidative stresses has been questioned by many authors (Van Assche and Clijsters 1990; Chaoui et al. 1997; Cuypers et al. 2000).

Activity of SOD decreased in low Zn plants, which was associated with higher accumulation of $O_2^{\cdot-}$ (r=-0.60) and MDA (r=-0.51) as indicator of membrane damage. Activity of SOD was suggested to be an indicator of Zn nutritional status of plants (Cakmak et al. 1997) and is the first enzyme activity known to be reduced under low Zn stress. In contrast, SOD activity increased in plants suffered from Fe deficiency. Induction of SOD activity by low Fe supply was reported by other authors (Molassiotis et al. 2006) and is attributable to production of more reactive oxygen species in Fe deficient plants.

Increase in the $O_2^{\cdot-}$ accumulation in Fe deficient plants was observed though activity of SOD did not reduced or rather increased (r=0.78). In means that, production of $O_2^{\cdot-}$ in Fe deficient plants was much higher than the capacity of scavenging enzymes. In contrast, change in the activity of SOD in Zn deficient plants was reflected well in the concentration of $O_2^{\cdot-}$ (r=-0.60).

In this work, responses of plants to low Zn and Fe as well as hypoxia stress are mainly attributable to the accumulation of $O_2^{\cdot-}$ than that of H_2O_2 or membrane damage. Detrimental effect of $O_2^{\cdot-}$ in comparison with H_2O_2 was well demonstrated in negative correlation between concentration of $O_2^{\cdot-}$ and plants dry weight (r=-0.79). Such correlation for H_2O_2 was rather positive (r=0.88-0.90). It means that like POD activity, H_2O_2 concentration has no determinant role in plants growth response. Superoxide radical is one of the most deleterious reactive oxygen species attacks membranes and induces peroxidation of lipids (Kappus 1985). It could be assumed that, tolerance to low Zn and Fe as well as hypoxia is highly related to the activity of superoxide radical scavenging system and particularly to the concentration of $O_2^{\cdot-}$ rather to the H_2O_2 or its scavenging enzymes. The ability to maintain a balance between the formation and detoxification of superoxide radicals appeared to increase the plants tolerance to low supply of tested nutrients as well as hypoxia. The importance of the superoxide scavenging system for maintaining the structural stability of subcellular plant organelles, integrity of cell membranes and protection of proteins, DNA and chlorophyll and deleterious effects of superoxide anion in cellular metabolism (Fridovich 1995) provide a good reason for this conclusion.

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