CHARACTERIZATION OF Zn EFFICIENCY IN IRANIAN RICE GENOTYPES II. INTERNAL UTILIZATION EFFICIENCY

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Summary. Four rice genotypes differing in Zn efficiency were selected to study the physiological mechanisms for different susceptibility of genotypes to Zn deficiency. It was demonstrated that Zn efficiency was not associated with flooding tolerance, so that flooding tolerance trait could be detected among both Zn-efficient and Zn-inefficient genotypes. Total and water soluble Zn concentrations in shoot and roots showed that Zn efficiency was not due to different inactivation of Zn in plant tissues. Zn-efficient genotypes showed a higher re-translocation of Zn from mature to younger just growing leaves, and responsiveness to low Zn supply was observed only in Zn-efficient genotypes. Activities of two important Zn-enzymes, SOD (superoxide dismutase) and ADH (alcohol dehydrogenase), were affected by both Zn nutritional status and flooding. However, changes in the activity of SOD in shoot were not associated with Zn efficiency response, but in roots reduction of SOD activity in Zn-inefficient as well as an increase in Zn-efficient genotypes were observed. Changes in the activity of ADH did not correlate either with Zn deficiency or flooding response of the genotypes studied.

Key words: ADH, Re-translocation, Rice, SOD, Zn efficiency, Zn deficiency, Use efficiency.

Abbreviations: ADH - alcohol dehydrogenase, HEPES - N-2-hydroxyethylpiperazin-N-2-ethanesulfonate, MES - 2-[N-morpholino]-ethanesulfonate, PVPP - polyvinylpolypyrrolidone, SOD - superoxide dismutase.

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INTRODUCTION

Zinc is known to have numerous functions in the physiology and biochemistry of higher plants. Zn is an integral component of the enzyme structure of approximately 300 enzymes, e.g. alcohol dehydrogenase (ADH) and Cu-Zn superoxide dismutase (Cu/ZnSOD) or it has catalytic functions, e.g. carbonic anhydrase (Marschner, 1995).

Zn deficiency limits the production of low land rice much more than deficiency of other micronutrients (Cayton et al., 1985). Genotypic variations in tolerance to Zn deficiency e.g. Zn efficiency was reported by some authors (Yang et al., 1994, Hajiboland, 2000).

The mechanisms involved in Zn efficiency of plants in general, and of rice in particular, are still poorly understood. In previous works on Zn efficiency mechanisms in the IR (International Rice Research Institute) rice genotypes, uptake efficiency as an important mechanism for Zn efficiency was not evaluated (Hajiboland, 2000).

In most instances, despite the significant differences in severity of leaf deficiency symptoms and reduction of dry matter yield between Zn-efficient and Zn-inefficient genotypes in response to low Zn supply, the genotypes did not differ in the concentration of Zn in shoots or roots (Cakmak et al., 1996). Therefore, it has been suggested that Zn-efficient and Zn-inefficient genotypes differ mainly in the physiologically active Zn within the plants, i.e. internal Zn utilization efficiency.

Internal use efficiency could be characterized by a higher physiologically active Zn level and/or efficient utilization of Zn pool in plants e.g. re-mobilization from mature to young growing leaves.

It was reported that most of the Zn content in leaves is associated with low molecular weight complexes and storage metaloproteins or can be found as free ions and insoluble forms in the cell wall (Marschner, 1995). Therefore, the water soluble Zn fraction is considered to be the physiologically active fraction and thereby a better indicator of Zn nutritional status than of total Zn content (Cakmak and Marschner, 1987).

Additionally, the activity of enzymes which can be directly affected by Zn has been suggested to be a diagnostic indicator of Zn nutritional status of plants, i.e. physiologically active Zn. For example, in wheat plants activity of Zn/Cu SOD was closely related with the sensitivity of genotypes to Zn deficiency (Cakmak et al., 1997).

It was reported that symptoms of Zn deficiency normally appear shortly after flooding (Van Breemen et al., 1980) and drainage alleviates deficiency symptoms. In connection with decreased Zn availability after flooding, two Zn containing enzymes, ADH and SOD are of special interest for the response of a distinct rice genotype to flooding conditions, considering particularly that both enzymes are involved also in the response to flooding. Flooding effect on plants represents a combined effect of

two stresses, oxygen deficiency and oxidative stress due disability of the scavenging system to metabolize the toxic active oxygen species (Foyer et al., 1994). Activity of SOD, catalyzing the disproportion of superoxide, changes not only in response to flooding (Yordanova et al., 2004), but can be affected also by Zn supply (Cakmak et al., 1997; Hajiboland, 2000). It was reported that in wheat plants the activity of Zn/Cu SOD was closely related with the sensitivity of genotypes to Zn deficiency (Cakmak et al., 1997).

Alcohol dehydrogenase, another Zn enzyme, is the major terminal enzyme of fermentation in plants and is responsible for recycling of NAD during anoxia. It has been suggested that ethanolic fermentation permits tight cytoplasmic pH regulation (Roberts et al., 1985) and a three-fold increase in ADH activity increased the anoxic survivability of seedlings from 8% to 87% in corn (Hwang and Van Toai, 1991). In rice, Moore and Patrick (1988) reported that in response to low Zn supply, root ADH activity decreased in plants grown in flooded soil, which was accompanied by a reduction in plant growth and Zn concentration.

Remobilization of a given nutrient varies greatly depending on plant species and genotype. Identification of genotypes with high remobilization rate from older to younger leaves is of increasing interest in selection and breeding of genotypes for high nutrient efficiency (Marschner, 1995).

Accordingly, in our work with rice genotypes differing in Zn efficiency, Zn distribution pattern among different parts of shoot could be different, and in efficient genotype it may be in favour of the young leaves as a result of higher remobilization from old to growing leaf initials. Therefore, higher internal utilization efficiency in terms of higher remobilization rate of Zn from old to young leaves could be one of the involving mechanisms in the higher Zn efficiency of a given genotype.

We have shown that rice genotypes with different Zn efficiency in terms of growth and chlorophyll concentration at low Zn supply, do not differ in Zn concentration in the shoot under deficient conditions (Hajiboland and Salehi, 2006). Therefore, higher internal utilization of Zn in efficient genotypes is one of the possible mechanisms.

In this work, using two Zn-efficient and two Zn-inefficient rice genotypes which were selected in a chelator-buffered nutrient solution experiment, the importance of internal utilization of Zn in the response of different rice genotypes to Zn deficiency was studied.

MATERIALS AND METHODS

Plant material and growth conditions

Four rice genotypes including Fajr, Tarom Hashemi, Shafagh and Amol were used in this work. Genotypes Fajr and T. Hashemi were characterized as Zn-inefficient while Shafagh and Amol were determined as Zn-efficient genotypes in a preliminary ex-

periment. Seeds were provided by the Research Center of Rice, Guilan province, Iran.

Growth conditions and plant cultivation were described elsewhere (Hajiboland et al., 2003). In summary, after germination, plants were pre-cultured in 25% and 50% nutrient solution (Yoshida et al., 1972) with low Zn (Zn<0.05 μM) for 6 days. Sixteen-day-old plants were transferred to treatment nutrient solutions either with low (<0.05 μM Zn) or adequate (0.5 μM Zn) Zn supply (pH 6.8). Nutrient solutions were completely changed every 4 days and pH was adjusted every day. Plants were not aerated during pre-culture or the different Zn treatments, unless otherwise mentioned. For comparison of growth under hypoxic and aerobic conditions plants were cultivated simultaneously in aerated and un-aerated nutrient solutions during pre-culture as well as treatment.

After growing in treatment solutions for 16 days, the plants were harvested. For determination of Zn content, oven-dried samples were ashed and Zn concentration was determined by atomic absorption spectrophotometry (AAS). Chlorophyll concentration was measured by the method of Moran (1982).

Determination of water soluble Zn

Leaf samples were stored at –20°C until drying. Freeze-dried samples were weighed and ground. Water soluble Zn was extracted from the samples in 1 mM MES buffer (pH 6.0) at continuous shaking for 5 h (Cakmak and Marschner, 1987). The samples were filtered and Zn concentration was analyzed by AAS.

Re-translocation experiment

Fourteen-day-old plants, which were pre-cultured in an 8 L container at low Zn (<0.05 μM) nutrient solution were used in this experiment. The nutrient solutions were substituted by Zn-loading solution with the same composition of pre-culture solution but buffered with 2 mM MES at pH 6.0 and Zn concentration (as $ZnSO_4$) of 1.0 μ*M*. After 48 h loading, the roots were washed with 0.5 mM $CaSO_4$ for 1h, and plant bundles after changing the holding sponges were transferred to 2.3 L dark pots with nutrient solution at low (<0.05 μM) and adequate (0.5 μM) Zn concentration. The experiment continued for 21 days and nutrient solutions were changed every 4 days (Hajiboland and Römheld, 2001).

Zn distribution among individual fractions of the shoot was studied at different harvest times, which were defined by the emergence and partial expansion of a new leaf. At the end of the experiment genotypes did not differ in the number of leaves but in the size of new leaves. The first harvest was performed at the beginning of Zn supply after root washing, and the following three harvests were carried out each time after a new leaf had emerged and partially expanded to a length of about 5 to 7 cm. Four replicates consisting of defined leaves of four plants were harvested for

each treatment, genotype and harvest time. Samples were weighed and washed with double distilled water, blotted on a filter paper and dried at 70 °C for 2 days for determination of dry weight. Finally, Zn was determined by AAS.

Assay of enzyme activities

Plants were rinsed with double distilled water and after drying on a filter paper and determination of fresh weight, they were used for extraction of enzymes. Total superoxide dismutase activity (SOD; EC 1.15.1.1) was determined according to Giannapolitis and Ries (1977). The enzyme was extracted in 25 mM HEPES (pH 7.8) and 0.1 mM EDTA, then the homogenate was centrifuged at 15 000 *g* for 15 min. Test tubes containing 25 µl of the enzyme extract, 25 µl extraction buffer and 450 µl of the reaction mixture were incubated in a growth chamber at 22°C and at light intensity of 400 µmol $m^{-2}s^{-1}$. The reaction buffer contained 25 mM HEPES (pH 7.6), 0.1 mM EDTA, 50 mM Na_2CO_3 (pH 10.2), 12 mM L-methionine, 75 µM NBT (*p*-nitro blue tetrazolium chloride) and 1 µM riboflavin. The reaction started by removing a dark plastic foil from the surface of the samples and continued for 10 min. One unit of SOD was defined as the amount of enzyme required to induce a 50% inhibition of NBT reduction measured at 560 nm, compared with control samples without enzyme aliquot.

Determination of alcohol dehydrogenase activity (ADH; EC 1.1.1.1) was carried out according to Pedrazzini and Mc Kee (1984). Fresh samples were homogenized in an ice container using pre-chilled mortar and pestle in 1 ml of extraction buffer per 200 mg fresh weight. The extraction buffer consisted of 50 mM cold HEPES buffer containing 5 mM $MgCl_2$, 2 mM cysteine hydrochloride and 2 % (w/v) of insoluble polyvinylpolypyrrolidone (PVPP) at pH 7.8. After centrifugation at 10 000 *g* for 10 min, the supernatant was used for determination of the enzyme activity. The assay solution (pH 8.0) contained 14 mM HEPES, 5.4 mM $MgCl_2$, 0.13 mM NADH. The reaction started by the addition of 4 mM acetaldehyde at 30°C. The enzyme activity was monitored as disappearance of NADH at 340 nm for 3 min. Determination of protein in the samples was carried out according to Bradford (1976).

Statistical analyses were carried out using Sigma Stat (3.02) and Tukey's test at p=0.05.

RESULTS

Growth and dry matter production

Plant growth was affected by low Zn supply to a different extent depending on the genotypes. The highest growth inhibition was observed in Fajr and T. Hashemi. In contrast, in Shafagh and Amol, low Zn supply did not affect shoot and root growth

significantly (Table 1). These results were in accordance with the results with a chelator-buffered technique reported elsewhere (Hajiboland and Salehi, 2006).

Hypoxic conditions in the nutrient solution did not affect shoot and root growth of the cultivars T. Hashemi and Shafagh, at both Zn treatments. In contrast, cultivars Amol and Fajr responded clearly to hypoxia in the nutrient solutions. Surprisingly, shoot as well as root growth of these two cultivars were significantly improved when grown in un-aerated nutrient solution (Table 1).

Hypoxia had different effects on chlorophyll concentration depending on the genotypes. A significant increase in chlorophyll concentration in response to hypoxia was observed in Amol at both Zn supplies. A reduction of chlorophyll concentration in response to hypoxia was observed in Shafagh at adequate Zn supply (Table 1).

The comparison of growth and chlorophyll data of the tested genotypes revealed that the order of hypoxia tolerance at low Zn supply was as follows: Amol>Fajr>T. Hashemi>Shafagh while at adequate Zn supply was the following: Fajr>Amol>T.Hashemi>Shafagh. Therefore, the order of hypoxia tolerance depended on the level of Zn. The Zn-inefficient cultivars were more tolerant to hypoxia if grown at adequate Zn supply.

Growth and chlorophyll data showed that two hypoxia the tolerant genotypes (Fajr and Amol) were highly contrasting genotypes in terms of Zn deficiency tolerance.

Total and water soluble Zn concentration

Zn concentration in shoot was higher in sufficient than deficient plants, but it was not affected by hypoxia treatment significantly. On the other hand, at low Zn supply, Zn

Table 1. Dry matter production and chlorophyll content of four rice genotypes grown in a nutrient solution at low (Zn<0.05 μM) or adequate (Zn=0.5 μM) Zn supply with or without aeration. Values in each column and row within each genotype and fraction followed by the same letter are not significantly different (P<0.05).

Genotypes	Treatment	Shoot DW (mg plant^{-1})		Root DW (mg plant^{-1})		Chlorophyll (mg g^{-1} FW)	
		–Aeration	+ Aeration	–Aeration	+ Aeration	–Aeration	+ Aeration
Fajr	Low Zn	68±8^{b}	45±5^{c}	12.1±2.3^{b}	8.7±1.8^{b}	1.73±0.18 ^{b}	1.42±0.11 ^{b}
	Adequate Zn	127±12^{a}	80±9^{b}	28.5±4.2^{a}	12.5±1.9^{b}	3.27±0.27 ^{a}	3.11±0.18^{a}
T. Hashemi	Low Zn	69±9^{b}	74±8^{b}	18.9±2.6^{b}	19.7±2.8^{b}	1.36±0.08 ^{b}	1.45±0.08 ^{b}
	Adequate Zn	169±18^{a}	163±11^{a}	32.7±4.4^{a}	29.2±3.0^{a}	2.12±0.11 ^{a}	2.54±0.11^{a}
Shafagh	Low Zn	65±9^{b}	63±8^{b}	12.3±4.1^{a}	10.4±3.8^{a}	2.45±0.35 ^{b}	2.55±0.23 ^{b}
	Adequate Zn	71±12^{ab}	85±9^{a}	14.3±3.7^{a}	12.9±1.4^{a}	2.88±0.27 ^{b}	3.45±0.24^{a}
Amol	Low Zn	133±1^{a}	88±9^{b}	36.4±4.9^{a}	19.6±4.1^{b}	3.04±0.22 ^{a}	2.49±0.12 ^{b}
	Adequate Zn	128±11^{a}	91±10^{b}	37.8±4.6^{a}	21.1±2.9^{b}	3.23±0.22 ^{a}	2.56±0.22^{b}

concentration of shoot in two Zn-inefficient genotypes (Fajr and T. Hashemi) was not lower than two Zn-efficient genotypes (Shafagh and Amol) (Table 2).

Water soluble Zn concentration showed the same tendency as total Zn concentration in the shoot. The proportion of water soluble Zn in total Zn was not different between Zn-efficient and Zn-inefficient genotypes. Treatments such as low Zn and hypoxia also did not affect this proportion.

Table 2. Concentration of total Zn, water soluble Zn (μg g^{-1}DW) and the ratio of water soluble/total (%) in four rice genotypes grown in a nutrient solution at low (Zn<0.05 μM) or adequate (Zn=0.5 μM) Zn supply with or without aeration. Values in each column and row within each genotype and fraction followed by the same letter are not significantly different (P<0.05).

Genotypes	Treatment	**Total**		**Water Soluble**		**%**	
		–Aeration	+ Aeration	–Aeration	+ Aeration	–Aeration	+ Aeration
Fajr	Low Zn	19.5±4.4 ^{b}	16.5±3.2 ^{b}	12.9±2.9 ^{ab}	9.6±0.51 ^{b}	0.66	0.57
	Adequate Zn	35.7±7.8 ^{a}	31.3±4.1 ^{a}	23.5±5.8 ^{a}	21.8±9.2 ^{a}	0.65	0.69
T. Hashemi	Low Zn	20.2±4.2 ^{b}	21.9±3.8 ^{b}	11.9±1.2 ^{b}	12.9±1.8 ^{b}	0.59	0.59
	Adequate Zn	39.3±8.9 ^{a}	43.2±2.9 ^{a}	24.4±3.2 ^{a}	26.8±8.9 ^{a}	0.62	0.62
Shafagh	Low Zn	19.2±7.8 ^{b}	18.8±2.9 ^{b}	12.5±2.7 ^{b}	10.4±3.2 ^{b}	0.65	0.55
	Adequate Zn	35.4±5.8 ^{a}	33.2±3.9 ^{a}	24.4±3.1 ^{a}	20.6±5.3 ^{a}	0.69	0.62
Amol	Low Zn	18.1±4.1 ^{b}	20.4±7.1^{b}	11.1±1.9 ^{b}	11.7±0.13 ^{b}	0.61	0.57
	Adequate Zn	39.9±5.4 ^{a}	40.4±5.3 ^{a}	23.8±2.4 ^{a}	24.52±9.7 ^{a}	0.59	0.60

Re-translocation of Zn

The amounts of Zn in the 1st and 2nd leaves, were continuously decreasing during the analyzed growth period (Table 3). This reduction in Zn content was observed in both deficient and sufficient plants, but to a different extent depending on Zn nutritional status and genotype.

Reduction of Zn content in the 1st and 2nd leaves between first and second harvest intervals in two Zn-efficient genotypes occurred though continuing growth, as was reflected in increasing weight of leaves (data not shown). Although Zn content in the 1st and 2nd leaves was also reduced in two Zn-inefficient genotypes, this began later, particularly it T. Hashemi, and occurred to a lower extent compared with the two Zn-efficient genotypes (Table 3). The reduction of Zn content in the 1st and 2nd leaves of cv. Fajr at adequate Zn was higher when compared with low Zn supply.

In contrast to the 1st and 2nd leaves, Zn content in the 3rd and 4th leaves decreased at low Zn supply only in the two Zn-efficient genotypes. In Zn-inefficient genotypes it rather increased during the growth period. At adequate Zn supply an increase in the Zn content in the 3rd and 4th and younger leaves during the analyzed growth period was observed, obviously due to Zn transport from the roots.

Table 3. Changes in Zn content (μg plant part^{-1}) of distinct leaves and roots of four rice genotypes differing in Zn efficiency during four harvest intervals (28 days). Plants were grown with low (Zn<0.05 μM) and adequate (Zn=0.5 μM) Zn supply after two days of Zn loading. Leaves are numbered in order of emergence.

Genotype	Harvest intervals	Low Zn				
		Leaf Number				Root
		1+2	3+4	5+6	7+8	
Fajr	1	15.89±2.23	—-	—-	—-	14.02±3.23
	2	14.20±0.31	4.07±0.55	—-	—-	10.55±0.63
	3	11.28±1.20	6.01±1.93	2.01±0.08	—-	8.26±2.66
	4	12.67±2.26	7.05±1.09	2.41±0.86	1.39±0.83	5.44±0.68
T. Hashemi	1	13.13±1.42	—-	—-	—-	18.57±0.23
	2	19.08±2.20	3.53±1.02	—-	—-	12.08±1.54
	3	12.98±0.87	4.19±0.45	4.99±2.98	—-	9.85±1.71
	4	9.48±0.99	4.47±0.19	8.27±1.17	1.48±0.02	9.24±1.94
Shafagh	1	13.27±2.09	—-	—-	—-	16.36±1.87
	2	9.95±0.18	11.72±0.63	—-	—-	6.96±0.59
	3	8.27±1.15	9.46±3.82	2.42±0.47	—-	5.91±0.84
	4	4.19±1.19	7.02±2.07	4.74±0.79	4.63±1.33	7.00±1.48
Amol	1	14.18±2.46	—-	—-	—-	16.10±1.28
	2	11.16±2.32	17.11±4.81	—-	—-	5.82±1.26
	3	6.91±1.18	15.50±2.14	5.31±1.21	—-	2.73±0.85
	4	5.05±1.01	7.69±0.44	8.67±1.45	5.47±0.34	5.07±1.18
Genotype	Harvest intervals	Adequate Zn				
		Leaf Number				Root
		1+2	3+4	5+6	7+8	
Fajr	1	15.89±2.23	—-	—-	—-	14.02±3.23
	2	13.82±2.79	5.72±0.97	—-	—-	4.39±0.64
	3	8.73±2.31	10.29±2.37	6.54±2.63	—-	4.71±0.87
	4	6.51±1.78	9.91±2.01	16.27±5.00	24.33±7.33	7.29±1.88
T. Hashemi	1	11.13±1.42	—-	—-	—-	15.57±0.23
	2	18.88±1.46	3.12±1.38	—-	—-	13.71±0.87
	3	16.97±1.96	10.96±0.93	4.69±0.98	—-	16.64±8.85
	4	9.55±0.93	10.69±1.61	15.40±13.36	17.73±0.43	7.78±2.82
Shafagh	1	13.27±2.09	—-	—-	—-	16.36±1.87
	2	12.58±1.08	2.11±0.40	—-	—-	2.54±1.54
	3	13.49±1.08	8.96±1.18	5.32±0.80	—-	5.02±0.51
	4	8.13±2.54	10.44±1.40	19.76±4.46	18.41±3.30	6.90±0.78
Amol	1	14.18±2.46	—-	—-	—-	16.10±1.28
	2	13.00±2.84	6.70±1.02	—-	—-	2.95±0.13
	3	8.67±2.42	5.93±2.07	5.17±1.87	—-	4.43±1.99
	4	7.57±0.17	6.66±0.66	17.76±3.56	8.96±3.12	6.96±1.88

Comparing the first and last harvest, two Zn-efficient genotypes grown at low Zn supply lost much more Zn quantity in the 1st and 2nd leaves than sufficient plants. However, the effect of Zn supply on the extent of re-translocation was not observed in the two Zn-inefficient genotypes. A clear genotypical difference could be also found for the 3rd and 4th leaves. In the Zn-inefficient genotypes a further increase of Zn content in the 3rd and 4th leaves took place (Table 3).

The root-derived Zn contributed also to the Zn content of shoot, which was clear from the decreasing amounts of Zn in this organ. The reduction (% or absolute) in Zn content in the root was similar (T. Hashemi and Shafagh) or rather higher (Fajr and Amol) in deficient than sufficient plants.

Total Zn content of plants at low Zn supply was generally constant throughout the analyzed growth period (data not shown). At adequate Zn supply, total Zn content increased during the experiment and it derived obviously from the nutrient solution.

Activity of Zn enzymes

Hypoxic conditions reduced significantly activity of SOD at both Zn supplies in all genotypes and in both shoot and root. In roots, reduction of SOD activity in response to flooding was not different between Zn deficient and sufficient plants. In shoots, however, the reduction was much higher in deficient than sufficient plants in all four tested genotypes. For example, in Amol up to 87% reduction in deficient compared with sufficient plants was observed.

Zn deficiency in root had different effect on SOD activity depending on the genotypes. In Zn-efficient genotypes, activity of SOD increased in response to low Zn supply, but decreased in Zn-inefficient genotypes. This effect was more pronounced in aerated than un-aerated plants. In shoots of aerated plants, however, similar to the two Zn-efficient genotypes, SOD activity increased in response to low Zn supply significantly (Fajr) or as a tendency (T. Hashemi). But in shoots of un-aerated plants, a significant reduction of SOD activity was also observed in the two Zn-efficient genotypes (Table 4).

Generally, hypoxic conditions decreased ADH activity in both shoot and particularly root in all tested genotypes at both Zn supplies significantly or as a tendency. Low Zn supply reduced ADH activity in shoot and root, but a correlation between the extent of reduction and Zn deficiency response was observed. For example, Amol and Shafagh with high Zn efficiency showed a greater inhibition of ADH activity than genotypes with lower deficiency tolerance. There was also a correlation between hypoxia tolerance and changes in the ADH activity under low Zn supply. Amol, a Zn-efficient and flooding tolerant genotype, showed a higher reduction of ADH than all other genotypes tested (Table 5).

Table 4. Activity of SOD (unit mg^{-1} protein) in four rice genotypes differing in Zn efficiency and flooding tolerance grown in aerated and unaerated nutrient solutions at low (Zn <0.05 μM) or adequate (Zn=0.5 μM) Zn supply. Values in each column and row within each genotype and plant part followed by the same letter are not significantly different (P<0.05).

Genotypes	Treatment	Shoot		Root	
		–Aeration	+ Aeration	–Aeration	+ Aeration
Fajr	Low Zn	46.2±18.0 ^{c}	307.5±58.2 ^{a}	68.6±21.6 ^{c}	243.2±15.7 ^{b}
	Adequate Zn	76.4±22.0 ^{bc}	157.5±43.7 ^{b}	78.1±9.2 ^{c}	351.6±80.1 ^{a}
T. Hashemi	Low Zn	154.8±34.4 ^{b}	286.6±54.8 ^{a}	100.1±44.3 ^{b}	345.4±148.3 ^{ab}
	Adequate Zn	163.6±41.4 ^{b}	231.2±13.3 ^{ab}	157.5±9.9 ^{b}	569.0±154.1 ^{a}
Shafagh	Low Zn	88.8±14.5 ^{c}	360.4±68.7 ^{a}	124.1±24.5 ^{c}	997.6±108.2 ^{a}
	Adequate Zn	205.2±10.6 ^{b}	242.5±11.0 ^{b}	69.4±7.5 ^{c}	423.9±121.1 ^{b}
Amol	Low Zn	47.5±12.8 ^{c}	369.4±15.8 ^{a}	141.7±15.7 ^{c}	535.8±82.7 ^{a}
	Adequate Zn	260.1±27.2 ^{b}	244.5±44.1 ^{b}	100.7±16.8 ^{c}	384.8±35.6 ^{b}

Table 5. Specific activity of ADH (μmol NADH min^{-1} mg $protein^{-1}$) in four rice genotypes differing in Zn efficiency and flooding tolerance grown in aerated and unaerated nutrient solutions at low (Zn <0.05 μM) or adequate (Zn=0.5 μM) Zn supply. Values in each column and row within each genotype and plant part followed by the same letter are not significantly different (P<0.05).

Genotypes	Treatment	Shoot		Root	
		–Aeration	+ Aeration	–Aeration	+ Aeration
Fajr	**Low Zn**	**13.9±5.3 ^{a}**	**16.4±8.6 ^{a}**	**21.5±4.7 ^{b}**	**49.4±1.0 ^{a}**
	Adequate Zn	13.0±2.2 ^{a}	16.3±5.7 ^{a}	45.1±5.7 ^{a}	59.2±7.5 ^{a}
T. Hashemi	**Low Zn**	**10.8±1.7 ^{a}**	**29.5±6.7 ^{a}**	**34.5±12.9 ^{a}**	**66.6±6.9 ^{a}**
	Adequate Zn	30.6±14.3 ^{a}	30.4±6.6 ^{a}	37.5±11.1 ^{a}	71.6±27.8 ^{a}
Shafagh	Low Zn	8.9±3.4 ^{b}	8.9±1.5 ^{b}	34.8±9.2 ^{b}	55.8±6.8 ^{b}
	Adequate Zn	13.3±1.7 ^{ab}	17.2±3.0 ^{a}	46.4±8.1 ^{b}	111.0±16.8 ^{a}
Amol	Low Zn	5.9±1.8 ^{c}	15.5±2.3 ^{ab}	27.9±2.8 ^{c}	76.2±27.9 ^{ab}
	Adequate Zn	13.5±2.9 ^{ab}	22.7±6.22 ^{a}	70.8±6.4 ^{ab}	148.4±66.5 ^{a}

DISCUSSION

Considering growth and chlorophyll data it was clearly shown that, Fajr and T. Hashemi could be ranked as Zn-inefficient, Shafagh and Amol as Zn-efficient genotypes. This is in accordance with the results of chelator-buffered nutrient solution experiments described elsewhere. Despite the considerable difference in growth

response between the two Zn-efficient and the two Zn-inefficient genotypes, Zn concentration in shoot and root was not different. Therefore, it seems most likely that the higher Zn use efficiency is involved in different responses of genotypes.

Relationship between flooding tolerance and Zn efficiency

Zn deficiency in lowland rice is mainly induced by water logging because of formation of insoluble Zn fractions in soils, such as Zn sulfide. Therefore, flooding tolerance and Zn efficiency in rice possibly have been developed simultaneously during natural and artificial selection for plants grown on water logged soils low in available Zn.

Chlorophyll and growth data of the four selected genotypes under flooding stress imposed by growth in nutrient solution without aeration showed a clear difference among genotypes in flooding tolerance. However, submergence tolerance was not necessarily associated with Zn efficiency trait. Fajr and Amol as two tolerant genotypes to flooding were contrasting genotypes in terms of Zn efficiency. Responses of plants to Fe and Mn toxicity as other components of flooding conditions were also in accordance with flooding, but not Zn deficiency responses (data not shown). However, in this work only one of the components of flooding tolerance e.g. hypoxia tolerance was studied. The oxidation and oxygenation power of roots, which play an important role for flooding responses of soil-grown rice plants (Begg et al., 1994), was not studied here. Therefore, the effect of such rhizosphere processes on efficiency response of genotypes could not be ruled out.

Relationship between the amount of bioavailable Zn in shoot and Zn efficiency

The water soluble Zn fraction is considered to be the physiological active fraction and thereby a better indicator of Zn status than total Zn content (Cakmak and Marschner, 1987). As Zn concentration in the various rice genotypes did not correlate with Zn efficiency (Hajiboland and Salehi, 2006), it was of interest to test the correlation of Zn efficiency with water soluble Zn. However, water soluble Zn did not correlate with Zn efficiency, too. Thus, it could be suggested that differential water soluble Zn may be a reliable indicator of bioavailable Zn under some treatments affecting Zn solubility and availability within plants (e.g., high phosphate or bicarbonate) but does not explain differential genotypical responses to Zn deficient conditions. No correlation between water soluble Zn and deficiency response of plants was also found for IR rice genotypes (Hajiboland, 2000).

Internal use efficiency in terms of Zn distribution pattern

A clear redistribution of Zn between older leaves and the youngest growing leaf could be shown during a long growth period with repeated harvest dates. Loaded Zn in the first two mature leaves was depleted rapidly during the following experimental period and this Zn was re-translocated to younger leaves as judged by the Zn depletion from the 1st and 2nd leaves simultaneouly with appearance of new leaves.

Additionally, in this work a clear genotypic difference was observed in the extent of re-translocation, e.g. re-translocation of Zn was more pronounced in the two Zn-efficient genotypes than the Zn-inefficient ones. The highest re-translocation (e.g. %reduction of Zn content) was 20% in Zn-inefficient (Fajr) genotype, while it was as high as 70% in the leaves of similar age and under the same treatment in both Zn efficient genotypes. Such a genotypic difference was also observed in two contrasting IR genotypes (Hajiboland and Römheld, 2001).

The re-translocation took place in the 1st and 2nd leaves of Zn-inefficient genotypes with a significant delay particularly in T. Hashemi, while a strong reduction of Zn content was obvious soon after the first harvest in the two tested Zn-efficient genotypes.

On the other hand, the net re-translocation was particularly expressed at low Zn supply during the growth period. Some authors suggested that mobility of a nutrient might vary strongly with the adequacy of supply of the nutrient: with a higher mobility at luxury supply and a lower mobility at lower or insufficient supply (Loneragan et al., 1976). In contrast, no differences were observed in the re-translocation of iron from primary leaves to the shoot apex in bean plants with different Fe nutritional status (Zhang et al., 1996). However, wheat plants showed a higher Zn remobilization from old leaves to generative organs under Zn deficiency than at adequate Zn supply (Pearson and Rengel., 1994). The data presented in this work showed that the rate of remobilization depended on Zn supply only in Zn-efficient genotypes. In Zn-inefficient genotypes, re-translocation of Zn from the 1st and 2nd leaves in sufficient plants was rather higher than (Fajr) or similar with (T. Hashemi) deficient plants. Similar results were obtained for the 3rd and 4th leaves. The lack of responsiveness to low Zn supply could be the most important factor determining Zn deficiency response of Zn-inefficient genotypes in this work.

As mentioned above, in efficient genotypes under adequate supply of Zn, content of the 3rd and 4th leaves even increased, while in low Zn plants these leaves lost a considerable amount of Zn. It could be possible that progressive mixing of root- and nutrient solution-derived Zn due to a long growth period in nutrient solution having adequate Zn, did not allow the indication of Zn re-translocation out of the 3rd and 4th leaves, i.e. a high Zn input combined with a low output.

Our results showed that remobilization of Zn had already started soon after the appearance of a new leaf (5-7 cm) in the second youngest leaf. At this time the

second youngest leaf could still be a sink leaf for assimilates because its dry weight was still increasing (data not shown), suggesting that Zn was translocated out from leaves as soon as elongation ceased but before the dry weight of leaves reached its maximum. A distinct time course for each leaf as a source of photosynthates as well as a source for Zn may indicate a mechanism for Zn re-translocation which takes place independent of assimilate flow out via phloem.

Internal use efficiency in terms of Zn bioavailability within the plant

Although nutrients disorder other than Zn was reported also to induce production of reactive oxygen species (ROS), Zn deficiency had much greater impact on the metabolism of reactive oxygen species. Zn not only protects membrane components by binding to sulfhydryl groups of proteins and phospholipids (Bettger and O'Dell, 1991), but also prevents lipid peroxidation through its role in superoxide radical metabolism (Cakmak and Marschner, 1988). Deficiency of Zn is reported to enhance superoxide radical generation (Cakmak and Marschner, 1988; Hajiboland, 2000) and decrease activities of superoxide dismutases (SODs), particularly Cu-Zn SOD that has Zn as its constituent (Vaughan et al., 1982). On the other hand, hypoxic and anoxic conditions under which rice was normally cultivated, maintain transition metal ions in a more or less reduced state, thus inducing formation of reduced oxygen species. The same conditions can arise within submerged plants (Hendry and Brocklebank, 1985). Therefore, maintaining or even increasing activity of SOD under Zn deficiency, not only indicates biochemical use efficiency, but also plays a direct role in tolerance to Zn deficiency stress.

Reduction in the activity of SOD in the roots in response to low Zn supply correlated closely with the Zn efficiency response of the tested genotypes. Differential changes in SOD activity in association with Zn efficiency was also reported for various IR rice (Hajiboland, 2000) and wheat genotypes (Cakmak et al. 1997).

A significant reduction of SOD activity in hypoxia treated plants was one of the most prominent responses observed in this work irrespective of Zn supply and genotypes and in both shoot and root. However, the reduction of SOD activity in response to flooding was not different among genotypes though a significant difference in flooding tolerance was observed.

A higher sensitivity of Zn deficient plants was observed to flooding conditions in terms of reduction in the activity of SOD in shoots. However, any correlation between Zn deficiency response of genotypes and changes in the activity of SOD in deficient flooded plants was observed. It should be noted that responses of plants to a higher production of ROS under low Zn supply and flooding is not reflected only by changes in the SOD activity. Maintaining a balance between the formation and detoxification of ROS, which determines the survival potential of plants could be indicated by the activity of all involved enzymes and concentration of antioxidants.

Beside the effect of flooding on the reduction of Zn availability in soils, the metabolic injury as a consequence of hypoxia could be one of the determining factors in survival of plants in flooded low Zn soils. It was 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 (Moore and Patrick, 1988).

In this work, exposure of plants to hypoxic conditions reduced the ADH activity in both shoot and root and in all genotypes tested. Although Zn nutritional status affected the response of ADH activity to flooding in shoots, this effect was different among genotypes independent of their Zn efficiency trait. In addition, a correlation between flooding tolerance and the extent of reduction in ADH activity in response to flooding was found in this work.

Activity of ADH in shoot and root of aerated Zn deficient plants decreased in both Zn-efficient genotypes, but remained unchanged or only slightly decreased in Zn-inefficient genotypes. Therefore, in contrast to SOD, ADH activity could be assumed neither as an indicator for Zn nutritional status nor a determining factor for efficiency response of genotypes. Earlier results on IR genotypes have also suggested that ADH activity is not an indicator of Zn nutritional status of plants (Hajiboland, 2000).

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