CHARACTERIZATION OF Zn EFFICIENCY IN IRANIAN RICE GENOTYPES I. UPTAKE EFFICIENY

R. Hajiboland* and S.Y. Salehi

Plant Science Department, University of Tabriz, 51666 Tabriz, Iran

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Summary. Zn deficiency is one of the most important micro-nutritional disorders in lowland rice. In this work, fourteen Iranian rice cultivars were studied in the field as well as in solution culture experiments to determine genotypic differences for traits characterizing Zn efficiency. A low uptake efficiency was observed in some (Onda and Kadoos) of the Zn-inefficient genotypes. A relatively high correlation (r=0.77) could be found between Zn content but not Zn concentration of shoot and dry matter production of genotypes. On the other hand, partitioning of applied Zn to soil was different between vegetative and generative parts of plants depending on genotypes. In Onda and Kadoos (Zn-inefficient) accumulation of Zn occurred in straw while in Nemat and Shafagh (Zn-efficient) the major portion of Zn taken up by plants was accumulated in seeds. A change measured in nutrient solution pH was higher in older than younger, in deficient than sufficient and in Zn-efficient than Zn-inefficient plants. It could be concluded that uptake efficiency, particularly in combination with a higher acidification potential of the rhizosphere, could be an important mechanism for the differences in studied rice genotypes.

Key words: rice, Zn efficiency, Zn deficiency, uptake efficiency.

Abbreviations: DW - dry weigth, RDW - root dry weight, FZA - free zinc activity, DTPA - diethylentriamine pentaacetic acid, MES - 2-[N-morpholino]-ethanesulfonate.

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* Corresponding author, e-mail: ehsan@tabrizu.ac.ir

INTRODUCTION

Zinc deficient soils are common in various climatic regions world-wide and Zn deficiency is one of the most important nutritional problems after macronutrients deficiency in crop production. Among cereals, rice, sorghum and maize are reported to have a high susceptibility to Zn deficiency, whereas barely, wheat, oat and rye are grouped as less sensitive species (Clark, 1990).

Zn deficiency has been identified as the most widespread micro-nutritional disorder of wetland rice. The disorder under wetland conditions is common on sodic, calcareous, organic and poorly drained soils (Cayton, et al., 1985). However, there is a great variation among rice genotypes to tolerate Zn deficiency (Yang et al., 1994a, Hajiboland, 2000).

The term “efficiency” refers to the ability of a genotype or species to grow under low supply of a given nutrient with less deficiency symptoms and less reduction in dry matter production compared to inefficient genotypes or species (Marschner, 1995). Differences in nutrient efficiency can occur for a number of reasons, i.e., might be related to uptake, transport and/or utilization within the plants (Marschner, 1995).

Mechanisms involved in Zn efficiency of rice plants have been studied by some authors. Some attempts were made to relate certain genotypical characteristics and growth parameters to the degree of responsiveness to Zn deficiency in rice genotypes. From these factors, some mechanisms such as differences in Zn uptake parameters (e.g. K_m and V_{max}) (Bowen, 1986), interaction effects with Fe and P (Cayton et al., 1985) as well as bicarbonate (Yang et al., 1994a, 2003, Hajiboland et al., 2003, 2005) and flooding (Moore and Patrick, 1988) have been studied.

Plant roots may influence the availability and uptake of mineral nutrients by different mechanisms such as the release of root exudates, protons, bicarbonate ions and ectoenzymes. These mechanisms are of particular importance under conditions of low nutrient availability. Increased release of root exudates in response to nutrient deficiency of P and Fe has been observed in many plant species (Jones and Darrah, 1994).

It was reported that the acidification of rhizosphere and consequently solubilization of Zn in the rhizoplane increase the availability of Zn for rice plants under flooding conditions (Kirk and Bajita, 1995). However, there are no reports on the inducing effect of Zn deficiency in the acidification of rhizosphere and on possible genotypical differences.

Previous works on IR and Chinese rice genotypes have shown that uptake efficiency is not based on involving mechanisms for Zn efficiency either in plants grown in soil or nutrient solution (Hajiboland, 2000). It was suggested that bicarbonate tolerance in terms of improved root growth and release of organic acids is one of the important factors which determine the response of rice genotypes to Zn deficiency (Hajiboland et al., 2003, Yang et al., 1994a, 2003).

In all studies on Zn efficiency mechanisms in rice, only the genotypes from IRRI and some from China and India have been used. The rice cultivation in Iran had its origin in more than thousand years ago, and there are many traditional rice cultivars in Iran, which were subjected to breeding programs and are adapted to local soil and climatic conditions without any mixing with foreign cultivars.

On the other hand, rice is the second important food crop in Iran, and there are 500,000 ha rice fields in North Iran with up to 2,708,333 t $yaer^{-1}$ rice production. Zinc deficiency has been reported since 2001 from lowland fields of North Iran, and after that, great amounts of Zn fertilizers are being used every year by farmers, without any attention to its environmental consequences. Therefore, selection of rice genotypes capable of growing in soils with low Zn availability can reduce fertilizer requirements and is an approach for sustainable and environmentally-friendly rice production.

Our previous study demonstrated that in contrast to IR and Chinese genotypes, bicarbonate tolerance was not associated with Zn efficiency trait in Iranian rice genotypes (Hajiboland and Salehi, 2006). However, the possible differences in uptake of Zn from soil or nutrient solution and involvement of some rhizosphere processes in Zn efficiency are not known.

Identification of genotypic differences of Iranian rice cultivars in tolerance to Zn deficiency using the most frequently cultivated genotypes and characterization of possible differences in uptake of Zn as mechanisms for Zn efficiency were the main objectives of this work.

MATERIALS AND METHODS

Plant material

Forteen genotypes Amol, Dasht, Fajr, Kadoos, Khazar, Mianeh, Neda, Nemat, Onda, Sahel, Shafagh, Tarom Deilamani, Tarom Hashemi and Tarom Ramazanali were used to characterize Zn efficiency traits in field and hydroponic culture experiments. Seeds were provided by the Research Center of Rice, Guilan province, Iran.

Plants culture, treatment and harvest

Field experiment: The field experiment was established in Sarve-Cola Village, Mazandaran Province (North Iran) on farm soil during the growing season of 2004. The soil was calcaric fluvisol according to the FAO classification, and fine loamy mixed calcareous termic molic xerofluvents according to the USDA classification. At first, the surface soil (top 30 cm) was collected in four replications for some chemical and physical analyses. The soil was air dried and sieved through a 2 mm screen. Soil pH (in water) was 6.5, 1.78% OC, with a texture of 27% sand, 36% clay and 37% silt (clayey loam) and DTPA (diethylentriamine pentaacetic acid) extractable Zn as available fraction was 0.68 mg kg^{-1}. The amount of total Zn measured in digested soil samples was 12.9 mg kg^{-1}.

Rice seeds were germinated in a humid and relatively warm room. Then they were transferred to nursery and grown for four weeks. The moisture content in the nursery soil was maintained at field capacity. One-month-old seedlings raised in the above nursery were uprooted and transplanted at 20×20 cm spacing into previously flooded plots (0.8×4.0 m) in the field. Basal application of 200 ug ha^{-1}N (as urea), 100 kg ha^{-1}K (as KCl) and 150 kg ha^{-1}P (as triple superphosphate) was made to all plots during the growth season. The experiment was conducted in a split-plot arrangement of treatments in a completely randomized block design with four replications and 5 plants per replication. The whole-plot treatment consisted of two Zn fertilizer treatments including Zn_0 (no Zn added, indicated in the text as –Zn) and Zn_1 (50 kg ha^{-1}, indicated in the text as +Zn). The Zn fertilizer was commercial zinc oxysulphate ($xZnO.xZnSO_4$), ~30% Zn). Plots were irrigated every two weeks during the vegetative growth and soils were kept submerged throughout the growth season, and hand-weeded regularly.

After three months growth (end of August), plants were harvested. They were cut at ground level and divided into straw and seeds with hand. Samples were dried in 70^{o}C for two days and weighed. After grounding, samples were used for Zn analysis.

Zn uptake was calculated for the above ground part of plants including straw and grains using the following formula: (Grain yield ×Zn concentration of grains) + (Straw yield× Zn concentration of straw) and was expressed as µg plant^{-1}.

Hydroponic experiment: A similar experiment was conducted in a culture solution. Plant culture and growth conditions were described elsewhere (Hajiboland et al., 2003). Prior to treatments, plants were pre-cultured in a 50% nutrient solution (Yoshida et al., 1972).

A chelator-buffered nutrient solution technique was used in order to reduce Zn contamination in the medium. Sixteen-day-old plants were transferred to chelator-buffered nutrient solution with the following composition (Yang et al., 1994b, slightly modified): (mM): NH_4NO_3 1.5, $CaCl_2$ 1.00, $MgSO_4$ 1.6, K_2SO_4 2.0, KH_2PO_4 0.1, and (µM): H_3BO_3 2.0, $MnSO_4$ 5.0, $CuSO_4$ 2.0, $(NH_4)_6Mo_7O_{24}$ 0.05 and Fe as $FeCl_3$ at 40 µM. The HEDTA (N-2-hydroxyethyl-ethylenediamine-N,N',N'-triacetate) concentration (100 µM) was chosen to give a 50 µM excess of HEDTA above the sum of the Cu, Fe and Mn concentrations. MES (2-[N-morpholino]-ethanesulfonate) was added at 5.0 mM to buffer pH, which was adjusted at 6.0 using KOH. The chemical activity of Zn^{2+} and other ions in the nutrient solutions was calculated using Version 2.0 of GEOCHEM-PC (Parker et al., 1995). Zn-HEDTA concentrations were 5.0 and 20 µM, and the calculated free Zn^{2+} activities (FZA) were 23 and 130 pM and calculated $pZn^{2+}(-\log[Zn^{2+}])$ were 10.65 and 9.89, respectively. Nutrient solutions were not aerated during pre-culture or the different Zn treatment, and were completely changed every 4 days.

After growing in treatment solutions for 16 days (32-day-old plants), the plants were harvested. Each bundle of plants was divided into shoots and roots, weighed and washed with distilled water, blotted dry on filter paper and dried at 70°C for 2 days to determine plant dry weight (DW). Zn uptake was calculated with the following formula:

Zn uptake = (Zn content of shoot + Zn content of root)/Root DW

Zn transport = (Zn content of Shoot)/Root DW and were expressed as мg g^{-1}RDW.

Determination of Zn and chlorophyll

Zinc concentrations were determined by atomic absorption spectrophotometry (AAS). Chlorophyll concentration was measured according to the method of Moran (1982).

Using the results of the above described experiment, six extremely contrasting genotypes were selected for the following experiment.

Changes in root medium pH

For a quantitative demonstration of changes in the pH of the growth medium, an experiment was conducted using six contrasting genotypes in a hydroponic medium adjusted to pH 6.5 only on the first day. A conventional nutrient solution medium (Youshida et al., 1972) was applied. The following one-week growth was without pH adjustment and the pH was monitored daily. After one week and simultaneous with the nutrient solution change, pH was adjusted and plants were grown for another one week.

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

RESULTS

Zn deficiency symptoms

In field-grown plants with no Zn fertilization, leaf deficiency symptoms developed only in Onda and T. Hashemi genotypes. However, stunted growth could be visually indicated in all genotypes with the exception of Mianeh and Amol. In plants grown in a chelator-buffered nutrient solution, the first visual leaf symptoms were observed in Zn-inefficient genotypes at FZA activity of 23 pM after 12 days of Zn-HEDTA treatment. In contrast, leaf symptoms did not develop in Zn-efficient genotypes, particularly in Mianeh and Amol at 23 pM of FZA and only plant height was slightly affected. Zinc-deficiency symptoms started as a reddish-brown discoloration of the third leaf and developed later on other younger and older leaves.

Growth and dry matter production

Zn fertilization for deficient soil increased both vegetative and grain yield of plants. However, the effect was more prominent for grain than vegetative yield, and sometimes the effect of Zn application was not significant, for example in Mianeh for both yield components. Interestingly, in Kadoos vegetative yield increased but grain yield rather decreased in response to Zn fertilization. In contrast, in Nemat, only grain yield was affected by the Zn fertilizer and dry weight of straw did not increase significantly. According to the calculated E amounts for both yield components, Mianeh and Amol were the most Zn-efficient genotypes while T. Hashemi and Onda were the most Zn-inefficient genotypes. Khazar was also tolerant to Zn deficiency (Table 1).

Table 1. Vegetative (g 5 plants^{-1}) and grain (g 5 plants^{-1}) yields of 14 rice genotypes grown on Zn-deficient soils with or without Zn application. Zn efficiency (E) was calculated as (–Zn/+Zn)100. Values in each row within each yield component followed by the same letter are not significantly different ($P<0.05$).

	Vegetative yield (g 5 plants^{-1})			Grain yield (g 5 plants^{-1})		
Genotypes	–Zn	+Zn	E	–Zn	+Zn	E
Fajr	143±25 ^{a}	178±36 ^{a}	**80**	16.69±1.53 ^{b}	20.05±1.94 ^{a}	**83**
T.Hashemi	128±12 ^{b}	181±9 ^{a}	**71**	17.23±1.18 ^{b}	23.11±1.23 ^{a}	**74**
Onda	124±12 ^{b}	162±23 ^{a}	**76**	18.05±4.16 ^{b}	24.72±1.17 ^{a}	**73**
T.Ramazanali	175±11 ^{b}	242±18 ^{a}	**72**	16.27±1.12 ^{a}	18.07±1.13 ^{a}	**90**
Sahel	119±19 ^{a}	140±24 ^{a}	**85**	17.14±0.88 ^{b}	22.30±0.70 ^{a}	**77**
Dasht	130±16 ^{a}	147±11 ^{a}	**88**	16.16±1.63 ^{a}	16.20±1.14 ^{a}	**99**
T. Deilamani	116±6 ^{b}	143±20 ^{a}	**81**	12.98±1.08 ^{b}	17.96±0.69 ^{a}	**72**
Neda	110±5 ^{b}	131±16 ^{a}	**84**	16.98±0.73 ^{b}	20.87±1.9 ^{a}	**81**
Kadoos	88±13 ^{b}	117±5 ^{a}	**75**	13.75±0.76 ^{a}	11.66±0.94 ^{b}	**117**
Nemat	88±22 ^{a}	110±25 ^{a}	**80**	13.49±0.76 ^{b}	16.15±0.58 ^{a}	**83**
Khazar	84±22 ^{a}	105±40 ^{a}	**80**	18.18±1.25 ^{b}	20.25±0.48 ^{a}	**90**
Shafagh	95±11 ^{b}	122±16 ^{a}	**78**	12.02±1.81 ^{b}	15.02±0.20 ^{a}	**80**
Amol	98±5 ^{a}	107±14 ^{a}	**91**	12.18±1.26 ^{b}	14.98±1.02 ^{a}	**81**
Mianeh	123±8 ^{a}	124±11^{a}	**99**	19.21±1.35 ^{a}	20.13±1.71 ^{a}	**95**

Using a chelator-buffered solution technique, the differences among genotypes appeared more distinctly. According to shoot DW data, Mianeh, Amol and Shafagh were the most efficient while Fajr, T. Hashemi and Onda the most inefficient genotypes. Responses of dry weight of root and chlorophyll concentration to Zn deficiency were also in accordance with changes of shoot dry matter (Table 2).

Though any increase in root dry weight, the root/shoot DW ratio was increased in Zn deficient plants which was the result of less inhibition of root than shoot growth under Zn deficiency. However, changes in the root/shoot ratio did not correlate with the deficiency response of genotypes.

Table 2. Dry matter production and chlorophyll content (relative amounts) in 14 rice genotypes grown in a chelator-buffered nutrient solution with low (FZA=23 pM) and adequate (FZA=130 pM) Zn supply. Zn efficiency (E) was calculated as (low/adequate Zn)100. Values in each row within each parameter followed by the same letter are not significantly different (P<0.05).

	Shoot DW (mg plant^{-1})			Root DW (mg plant^{-1})		Chlorophyll (% over control)	Root/Shoot	
Genotypes	low	adequate	E	low	adequate		low	adeq.
Fajr	123±13 ^{b}	302±8 ^{a}	**41**	71±8 ^{b}	104±6 ^{a}	85.3±2.4	0.55	0.34
T.Hashemi	**149±12 ^{b}**	**355±18 ^{a}**	42	**67±6 ^{b}**	**110±7 ^{a}**	**81.1±2.2**	**0.45**	**0.30**
Onda	212±20 ^{b}	363±20 ^{a}	**58**	74±16 ^{b}	119±13 ^{a}	93.4±5.1	0.34	0.32
T.Ramazanali	**363±10 ^{b}**	**576±30 ^{a}**	63	**144±4 ^{b}**	**208±13 ^{a}**	**87.3±2.8**	**0.39**	**0.36**
Sahel	302±10 ^{b}	463±50 ^{a}	**65**	138±10 ^{b}	202±21 ^{a}	91.4±4.4	0.45	0.43
Dasht	269±30 ^{b}	408±20 ^{a}	**66**	127±17 ^{b}	180±5 ^{a}	92.8±2.0	0.46	0.43
T. Deilamani	**326±8 ^{b}**	**431±61 ^{a}**	76	**131±-5 ^{a}**	**119±15 ^{a}**	**85.8±3.4**	**0.39**	**0.27**
Neda	231±19 ^{b}	294±6 ^{a}	**79**	112±7 ^{b}	126±4 ^{a}	94.4±2.5	0.50	0.42
Kadoos	265±11 ^{b}	334±24 ^{a}	**79**	104±8 ^{a}	102±12 ^{a}	88.8±0.6	0.39	0.29
Nemat	374±40 ^{b}	450±20 ^{a}	**83**	167±14 ^{b}	200±17 ^{a}	90.4±1.0	0.44	0.44
Khazar	455±30 ^{b}	531±40 ^{a}	**86**	142±14 ^{b}	167±3 ^{a}	90.6±2.6	0.30	0.30
Shafagh	342±50 ^{a}	369±10 ^{a}	**93**	131±25 ^{a}	133±6 ^{a}	97.2±0.7	0.38	0.35
Amol	329±12 ^{a}	334±3 ^{a}	**99**	178±13 ^{a}	158±8 ^{a}	90.7±0.2	0.53	0.49
Mianeh	468±23 ^{a}	432±32 ^{a}	**108**	226±15 ^{a}	245±24 ^{a}	94.9±2.5	0.47	0.48

Between two experimental systems, an acceptable accordance for many of studied genotypes in terms of tolerance to Zn deficiency could be observed. Considering results of both field and solution culture experiments, Onda and T. Hashemi could be ranked as Zn-inefficient while Amol and Mianeh as Zn-efficient genotypes. The two other genotypes, Fajr and Shafagh, which demonstrated a high contrasting response only in the nutrient solution experiment, were also studied together with the four above-mentioned genotypes in the following experiments in this work.

Concentration and content of Zn

In the solution culture experiment, significant reduction of Zn concentration (µg g^{-1}DW) was observed in all genotypes at low Zn (Table 3). However, the extent of reduction of Zn concentration was not higher in the susceptible compared with the tolerant genotypes. On the other hand, in some genotypes for example in Onda, Zn concentration was not significantly different between adequate and low supply of Zn. In contrast, when grown at low Zn, content of Zn (µg plant^{-1}) in inefficient genotypes was lower than that of the efficient genotypes. Genotypes when grown with sufficient Zn did not differ in shoot Zn content (Table 3). However, high Zn content at low Zn supply was observed in Onda (inefficient). Content as well as concentration of Zn in the Shafagh genotype were also higher compared with the other studied

Table 3. Zn content and concentration in seeds and shoots of hydroponically-cultured 14 rice genotypes differing in Zn efficiency and grown at low (FZA=23 pM) or adequate (FZA=130 pM) Zn supply. Values of seed Zn of different genotypes followed by the same letter are not significantly different ($P<0.05$).

Genotypes	Zn content (μg plant^{-1})		Zn concentration (μg g^{-1} DW)		Seed	
	low	adequate	low	adequate	(μg g^{-1}DW)	(ng seed^{-1})
Fajr	14.0±2.7	60.7±6.6	389±44	566±38	14.1±2.8 ^{c}	331±64 ^{c}
T.Hashemi	15.1±0.6	52.4±3.0	294±24	440±40	7.4±3.7 ^{d}	165±82 ^{d}
Onda	50.9±4.9	79.5±8.9	676±56	661±103	27.2±9.7 ^{b}	759±182 ^{b}
T.Ramazanali	26.7±1.9	56.1±12.7	205±11	287±58	21.8±1.4 ^{b}	483±31 ^{b}
Sahel	21.2±1.3	73.4±3.9	194±4	509±41	58.8±9.9 ^{a}	1705±288 ^{a}
Dasht	51.7±14.8	86.6±10.9	547±93	639±52	49.2±19.4 ^{a}	1412±556 ^{a}
T. Deilamani	18.5±1.7	71.9±7.2	170±12	509±52	20.8±2.5 ^{b}	482±54 ^{b}
Neda	15.5±1.6	44.3±4.8	195±14	470±46	13.2±1.1 ^{c}	338±27 ^{c}
Kadoos	20.6±2.4	45.2±6.3	237±41	405±64	14.15±1.6 ^{c}	375±39 ^{c}
Nemat	20.9±3.4	68.2±2.1	166±13	463±26	18.2±8.8 ^{bc}	487±235 ^{bc}
Khazar	23.3±1.0	80.6±16.6	157±9	481±95	21.7±12.4 ^{bc}	581±332 ^{bc}
Shafagh	54.2±7.5	56.1±12.7	521±76	1170±103	14.4±3.6 ^{c}	279±70 ^{c}
Amol	26.3±2.3	73.4±3.9	241±14	521±86	11.0±1.0 ^{c}	213±20 ^{c}
Mianeh	23.2±1.1	86.6±10.9	148±16	546±177	12.3±1.0 ^{c}	433±35 ^{c}

genotypes not only in nutrient solution- but also in field- grown plants at all Zn treatments.

After elimination of Onda and Shafagh because of the extremely high Zn content, the statistical analysis showed that there was no significant correlation between shoot DW and Zn concentration under various Zn supply (Fig. 1-a). In contrast, a relatively high correlation (r=0.77) could be found between Zn content of shoot and dry matter production of plants under various Zn supply (Fig. 1-b).

Both Zn content and concentration of seeds also differed among genotypes, but independent from their efficiency trait. A high Zn content and concentration in seeds was observed in Onda, Sahel and Dasht (Zn-inefficient), but a relatively low Zn amounts were observed in Mianeh and Amol (Zn-efficient). However, lower seed Zn content was associated with intolerance to Zn deficiency in T. Hashemi.

Zn uptake and transport

In order to compare genotypes for their uptake efficiency, the results of both field and solution culture experiments were shown in Table 4. The lowest Zn uptake in field-grown plants without Zn fertilization was observed in Onda and Kadoos while the highest Zn uptake was in Shafagh. Such a high Zn uptake (and transport) was observed for the last genotype in a chelator-buffered nutrient solution. A relatively high Zn uptake was also observed in a chelator-buffered nutrient solution for Onda.

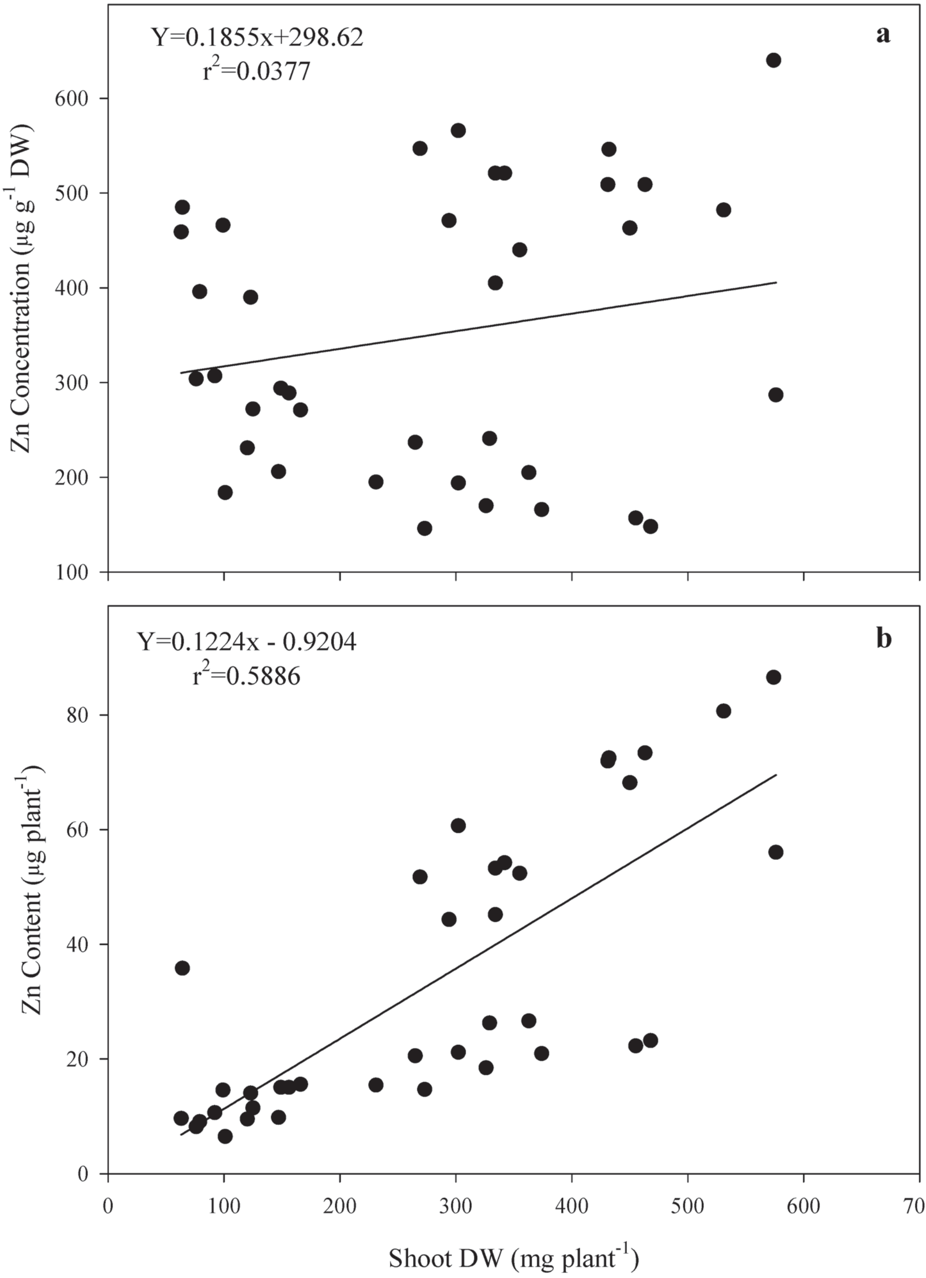

Fig. 1. Correlation between Zn concentration (g g^{-1}DW) or content (g plant^{-1}) and shoot dry matter production (mg plant^{-1}). Data are from three different Zn supplies including 130, 23 and 5 pM of free Zn activity for all studied genotypes with the exception of Onda and Shafagh.

Table 4. Uptake of Zn (μg plant^{-1}) in field-grown plants and uptake (μg g^{-1}RDW) and transport (μg g^{-1}RDW) in plants grown in a chelator-buffered nutrient solution with low (FZA=23 pM) and sufficient (FZA=130 pM) Zn supply. Values in each row between two Zn treatments and within each parameter followed by the same letter are not significantly different (P<0.05).

Genotypes	Field experiment		Nutrient solution experiment			
	Uptake (μg plant^{-1})		Uptake (μg g^{-1} RDW)		Transport (μg g^{-1} RDW)	
	–Zn	+Zn	low	adequate	low	adequate
Fajr	1652±64^{b}	3430±197^{a}	367±51^{b}	805±140^{a}	198±34^{b}	584±111^{a}
T.Hashemi	1303±20^{b}	2608±21^{a}	390±17^{b}	691±75^{a}	225±10^{b}	476±50^{a}
Onda	730±12^{b}	1532±18^{a}	1364±54^{a}	1089±82^{b}	689±31^{a}	668±69^{a}
T.Ramazanali	1303±10^{b}	2173±18^{a}	330±101^{a}	438±63^{a}	185±49^{a}	269±98^{a}
Sahel	1041±159^{a}	1220±84^{a}	299±28^{a}	564±34^{a}	153±13^{a}	363±18^{a}
Dasht	2369±73^{a}	2306±50^{a}	806±129^{a}	895±101^{a}	407±87^{a}	481±218^{a}
T. Deilamani	1791±31^{b}	2350±102^{a}	254±59^{b}	832±69^{a}	141±33^{b}	605±48^{a}
Neda	1188±6^{b}	3627±17^{a}	260±52^{b}	541±98^{a}	138±22^{b}	352±121^{a}
Kadoos	466±10^{b}	7204±4^{a}	344±36^{b}	673±69^{a}	198±31^{b}	443±53^{a}
Nemat	1385±121^{a}	1388±146^{a}	237±29^{b}	527±20^{a}	125±24^{b}	341±12^{a}
Khazar	1482±213^{b}	2739±387^{a}	282±12^{b}	653±61^{a}	164±7^{b}	483±154^{a}
Shafagh	4815±44^{a}	4035±64^{b}	615±41^{b}	1687±257^{a}	414±30^{b}	1135±154^{a}
Amol	1466±13^{b}	1832±33^{a}	258±26^{b}	495±114^{a}	148±17^{b}	337±54^{a}
Mianeh	1654±16^{b}	3045±25^{a}	188±27^{b}	441±29^{a}	103±7^{b}	296±23^{a}

It could be interesting to know how Zn taken up by the fertilized plants was partitioned into straw and grain. Considering Zn content (μg per organ) of each component presented in Fig. 2, it could be found that there were considerable genotypic differences in Zn partitioning between these two sinks. From all 14 genotypes studied, the most contrasting response was observed in Kadoos and Nemat (Fig. 2). In Kadoos, Zn fertilization resulted in a significant increase of Zn content in straw while only a non-significant increase in seeds was observed. Similar results were obtained in Onda with high Zn accumulation in dry weight of straw and rather a reduction of Zn content in seeds. The highest positive response of seed Zn to Zn fertilization was observed in Nemat with a reduction of Zn content in straw and an increase in seeds. The other genotypes showed a similar trend of an increase of Zn content in straw and seeds.

Changes in growth medium pH

The reduction of pH in the nutrient solution was higher in older plants, e.g. during the second week of growth. On the other hand, this reduction depended highly on Zn nutritional status of plants, particularly in Zn efficient genotypes. This effect was more pronounced in Amol. Acidification of the nutrient solution by this genotype was much higher (1.5 times) at low than adequate Zn supply. However, in Zn-ineffi-

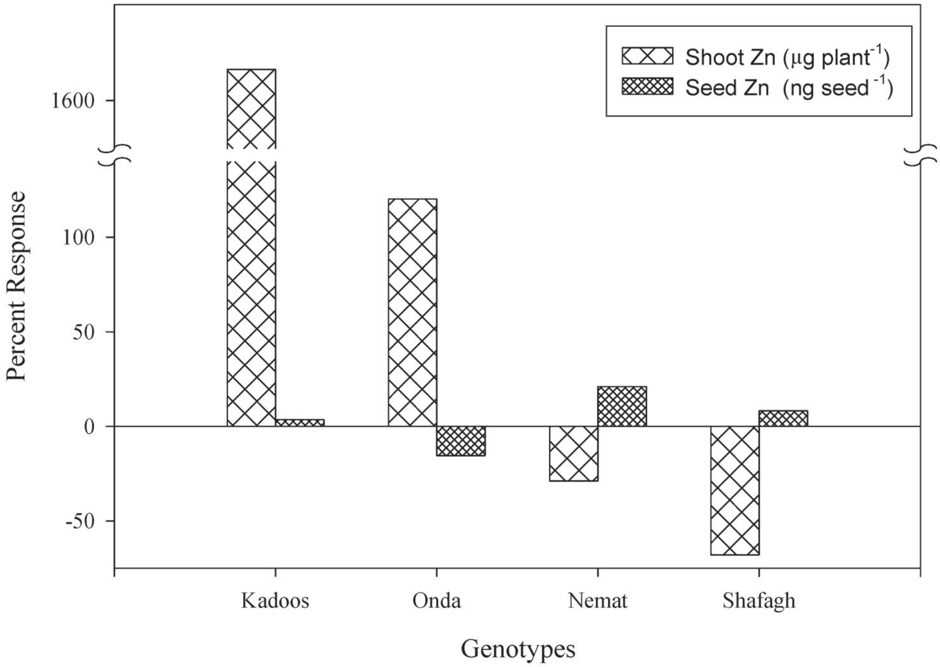

Fig. 2. Different responses (% over control) of Zn content in shoot and seeds to Zn fertilization in four rice genotypes grown in low Zn soils.

cient genotypes, the effect of Zn nutritional status on acidification of the growth medium was different from that of Zn-efficient genotypes. In Fajr, rather an adverse effect was observed, e.g. higher acidification in Zn-sufficient and lower in Zn-deficient plants (Table 5).

Table 5. Changes in nutrient solution pH (pH 6.5) induced by rice genotypes with different Zn efficiency during two weeks growth at low (Zn <0.05 μM) or adequate (Zn=0.5 μM) Zn supply without pH adjustment. Values in each column within each Zn level followed by the same letter are not significantly different (P<0.05).

Genotypes	**1st week of growth**		**2nd week of growth**	
	low	adequate	low	adequate
Fajr	4.36±0.08 ^{a}	3.67±0.12 ^{c}	3.77±0.06 ^{a}	3.31±0.12 ^{d}
T. Hashemi	4.42±0.09 ^{a}	4.38±0.07 ^{b}	3.90±0.05 ^{a}	3.98±0.07 ^{b}
Onda	4.45±0.07 ^{a}	4.58±0.04 ^{b}	3.99±0.03 ^{a}	4.05±0.03 ^{b}
Shafagh	3.80±0.09 ^{b}	3.78±0.11 ^{c}	3.39±0.05 ^{b}	3.62±0.12 ^{c}
Amol	3.95±0.08 ^{b}	5.60±0.09 ^{a}	3.00±0.08 ^{b}	4.55±0.05 ^{a}
Mianeh	3.92±0.01 ^{b}	4.12±0.05 ^{b}	3.33±0.03 ^{b}	3.93±0.02 ^{b}

DISCUSSION

In this work, some mechanisms involved in Zn efficiency of rice genotypes, including different uptake and transport and change in the rhizosphere pH were studied. Because ranking of genotypes using only the results of nutrient solution experiment might not be reliable, a field trial was also carried out in order to compare and evaluate the results of the culture solution experiment.

Characterization of Zn efficiency trait of plants

In field grown plants, vegetative and grain yield of genotypes increased mainly to a similar extent. However, in some genotypes grain yield did not respond prominently to Zn fertilization, but straw yield increased significantly and in some other genotypes, the opposite was observed. Considering responses of generative growth in terms of grain yield and Zn accumulation in seeds in response to Zn fertilization, four contrasting genotypes could be introduced in this work which are interesting model genotypes to study Zn mobilization from roots or leaves to filling grains. Because of nutritional importance of grain Zn for human, the role of leaves in supplying Zn for grains was studied in wheat (Pearson and Rengel, 1994). A higher grain loading of Zn could be one of other important aspects for breeding crops, such as wheat and rice for Zn efficiency trait.

Since Zn bioavailability is limited by low Zn mobility, and thus spatial availability in the soil solution (Marschner, 1995), species or genotypes having longer and finer roots, may extract more Zn from soils. However, in this work, significant difference among genotypes in root weight and length neither under control conditions nor in response to Zn deficiency was observed. Consequently, morphology of root system had a role in the determination of response of studied genotypes to Zn deficiency. In contrast to wheat (Cakmak et al., 1996), there has been no report of root growth induction under Zn deficiency in rice plants.

Differential susceptibility of rice genotypes to Zn deficiency did not correlate with Zn concentration or % reduction of Zn concentration in shoot and root. In contrast, Zn content was closely related to differences in sensitivity to Zn deficiency. The same results were obtained for wheat and some other cereals (Graham et al., 1992, Rengel and Graham, 1995, Cakmak et al., 1996) and also for rice (Hajiboland, 2000) grown under Zn deficiency.

Uptake efficiency as a factor involved in Zn efficiency

In field- as well as solution culture-grown plants, differential uptake of Zn was observed in the genotypes studied. A low uptake efficiency most likely was involved in the susceptibility of Onda and Kadoos to Zn deficiency when grown in soil. Higher Zn uptake from the nutrient solution did not change the deficiency response of Onda.

This genotype had a low Zn use efficiency not only at vegetative growth, but also for grain yield production.

Our results showed that Zn efficiency was not due to a different Zn translocation to shoot. Similarly, in wheat and bean cultivars differing in Zn efficiency no correlation between ^{65}Zn translocation into shoot and differential Zn efficiency was found (Hacisalihoglu, 2002). In contrast, for IR genotypes, it was found that, maintaining adequate Zn content in roots and a relatively low ^{65}Zn translocation into shoot at low Zn supply were likely one of the important criterion for determining low susceptibility of rice genotypes to Zn deficiency (Hajiboland, 2000).

Relationship between acidification potential of the rhizosphere and Zn efficiency

Soil pH is one of the important chemical factors affecting Zn availability for plants. As soil pH increases, Zn availability for plants decreases and Zn is adsorbed by soil constituents or precipitated by specific compounds (Marschner, 1995). Root-induced pH changes in the rhizosphere which could influence the availability of Zn and other nutrients are quite common (Marschner, 1995).

It was reported that the rhizosphere of rice plants growing in anaerobic soil is greatly acidified (Begg et al., 1994; Kirk, and Bajita 1995; Saleque and Kirk 1995). However, there is no information on the changes of pH occurring in the growth medium in response to low Zn supply in rice plants. It is likely that a shift of cation:anion uptake in favour of cations might occur in rice plants fed with NO_3^-+NH_4^+ with low Zn supply.

In this work, a significant reduction of nutrient solution pH was observed in all tested genotypes. This acidification potential was significantly higher in Zn-efficient than Zn-inefficient genotypes. On the other hand, a response to low Zn supply was observed only in the Zn-efficient genotypes. For example, the changes in pH was up to 3.5 units during one week in Amol when grown at low Zn supply.

In IR rice genotypes though the high accumulation of organic acids in roots, the release into rhizosphere was not significantly induced by Zn deficiency (Hajiboland, 2000). A significant release of organic acids could be demonstrated only in the presence of bicarbonate (Hajiboland et al., 2005), therefore, it could play a role in solubilization of Zn fractions only in plants grown in calcareous soils.

This is the first report on the genotypic differences in acidification of rooting medium in rice and its involvement in Zn efficiency. Root-mediated processes that can lower the rhizosphere pH increase the plant Zn availability by solubilizing Zn from organic and inorganic soil solid phase (Hacisalihoglu and Kochian, 2003).

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