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Zinc and Salinity Interaction on Agronomical Traits, Chlorophyll and Proline Content in Lowland Rice (*Oryza sativa* L.) Genotypes

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Abstract: Zinc deficiency in crop plants has been recognized as a worldwide nutritional constraint. A greenhouse experiment was conducted in order to evaluate the effects of Zn nutrition and salinity stress and their interaction on agronomical traits, chlorophyll and proline content of rice plant. Eight local and improved low land rice genotypes were grown at 0, 10 and 20 mg Zn kg^{-1} soil under saline (6 dS m^{-1}) or non-saline condition at tillering stage. Results showed that interaction between Zn×salinity effects on filled grains, 100 grain weight and chlorophyll content were widely varied in rice plant genotypes. Only 100 grain weight was significantly differ at Zn×salinity×genotype interaction levels. Free proline content was significantly lower in Zn-efficient genotypes (Shafagh, Pokkali, IR9764 and IR9884) in comparison with Zn-inefficient genotypes (Hashemi, Domsiah, Kados and IR26). It seems that under salinity stress, the higher content of free proline could be considered as an injury indicator in rice plant. It could be concluded that Zn-efficient rice genotypes tolerated salinity better than the Zn-inefficient genotypes.

Key words: Zinc, salinity, agronomical traits, proline, rice

INTRODUCTION

Rice is the major staple food for more than two billion people in Asia, Africa and Latin America. Of the total calories consumed globally, 23% are supplied by rice (Khush, 2001).The production and cultivation areas of rice are greatly menaced by soil salinity. Furthermore, much of the Iranian rice fields widely exposed to salinity stress are also deficient in zinc (Zn). The high pH conditions and high calcium concentration in most saline soils are responsible for the low availability of zinc and the occurrence of low production in crops in such soils (Alloway, 2004). One of the probable biochemical reactions in plants exposed to salinity stress is the production and accumulation of Reactive Oxygen Species (ROS). Zinc is required for scavenging of ROS including super oxid radical and (O_2^-) hydrogen peroxide (H_2O_2). Because of the Zn major role in the activation and expression of genes (Cakmak, 2000), this suggests a possible link between zinc deficiency and susceptibility to salinity stress. In addition, Zn efficient genotypes may show a higher salinity tolerance than Zn inefficient genotypes. Zn efficiency could be defined as the ratio dry matter production of plant grown under deficient and adequate Zn supply and is an indicator of genotypic tolerance to low supplies of Zn. Very few studies have been focused on the relationship between Zn nutrition and salinity tolerance in plants till now and much of investigations have been confined primarily on the effect of Zn ions on plant at seedling stage and *in vitro* condition. In the present experiment, the effects of zinc application and salinity stress on agronomical traits, one osmoprotectant (proline), Chlorophyll Content (CC) and the amount of zinc, was evaluated in mature plants of eight rice genotypes.

MATERIALS AND METHODS

Pot experiment: Eight genotypes of rice plant (*Oryza sativa* L.) were used in this experiment. Four genotypes were provided from IRRI (Philippines). Three Zn-efficient genotypes (Pokkali, IR 9884 and IR9764) and the other Zn-inefficient (IR26) used as control. Four Iranian varieties, Shafagh (improved variety/Zn-efficient) and Kados (improved variety/Zn-inefficient), Domsiah and Hashemi (local variety/Zn-inefficient) genotypes were obtained from Rice Research Institute of Iran. Plants were grown in a Zn-deficient sandy soil and basal nutrients (NPK) were added. Zinc treatments were 0, 10 and 20 mg Zn kg^{-1} soil applied as $ZnSO_4$, $7H_2O$. Four pre-germinated seeds from each of rice genotypes were sown in pots containing 5 kg soil. The experiment was carried out in a completely randomized block design with 4 replications. Each pot was immersed in a water filled

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bucket at the depth of 3-5 cm for two weeks after sowing. Salinity treatments (0 and 6 dS m^{-1}) were applied at the tillering stage till maturity. Panicle number, panicle length, plant height, total number of filled grains and 100 grain weight recorded at the end of plant growth.

Measurement of the chlorophyll content: Chlorophyll Content (CC) was measured by chlorophyll meter (Minolta, SPAD-502, Japan). The 2nd leaf from the shoot apex was measured at 4 positions for the CC at booting stage. The CC was calibrated with a standard curve of total chlorophyll concentration according to Shabala *et al*. (1998).

Zinc measurement: Half of the rice plants were harvested at booting stage and separated into shoot and root. Shoot tissues were dried for three days at 70°C. Then were ground and 0.3 g dry matter of each replicate was digested in 3.5 mL of acid mixture (H_2SO_4, Salicylic acid) at 180°C for 1 h, then cooled at room temperature. One milliliter of 30% H_2O_2 was added to the samples and heated at 180°C for 20 min. Then were cooled again. After suitable dilution, the Zn content of the samples were measured by Atomic Absorption spectrophotometer.

Determination of the proline content: Proline was extracted according to the Bates (1973). 250 mg of fresh weight were ground in a mortar with liquid nitrogen. The homogenate was mixed in 5 mL aqueous sulfosalisylic (3%w/v) acid and filtered through Whatman No. 1 (Whatman, England). The filtrate, 1 mL, was reacted with an equal volume of glacial acetic acid and ninhydrin reagent (1.25 mg of ninhydrin, 30 mL of glacial acetic acid and 20 mL of 6 M H_3PO_4) and incubated for 1h at 100°C in water bath. The reaction was terminated by placing the test tubes in an ice bath. The reaction mixture was vigorously mixed with 2 mL toluene. After warming at 25°C, the chromophore was measured for proline at 520 nm with a 6105 uv/vis spectrophotometer (JENWAY, England). The proline content was determined using a standard curve of 0-512 μmol of L-proline (Fluka, swizerland).

RESULTS

The results of the analysis of variance for the measured variables showed significant effect of Zn treatment on chlorophyll content, panicle number, panicle length, total number of grain and 100-grain weight

Table 1: Significance of F values derived from analysis of variance for variables measured on eight rice genotypes at three levels of zinc and two levels of salinity

Variable	Zn	Salinity (S)	Zn×S	Genotypes (G)	Zn×G	S×G	Zn×S×G	CV (%)
Chlorophyll content	**	**	*	*	ns	**	ns	22.12
Plant height	ns	**	ns	**	ns	**	ns	11.22
Panicle number	**	ns	ns	ns	ns	ns	ns	44.07
Panicle length	**	**	ns	**	ns	**	ns	7.5
Total number of grain	**	**	ns	**	ns	*	ns	17.08
Total filled grain	ns	**	**	**	ns	ns	ns	35.93
100-Grain weight	*	**	**	**	**	*	**	5.58

*, ** significant at the 5 and 1% levels, respectively, ns=not significant

Table 2: Changes in the studied traits under saline/non-saline conditions

Salinity (dS m^{-1})	Chlorophyll content	Plant height (cm)	Panicle length (cm)	Total No. of grain	Total filled grain	100-grain weight (g)
0	26.83a	98.39a	24.09a	82.81a	63.18a	2.21a
6	21.00b	88.49b	21.68b	67.87b	22.58b	1.90b

Different letter(s) (a and b) indicates that the difference between data are significant at 5%, The values represent the mean of four replications

Table 3: Changes in the studied traits at different Zn levels

Zn (mg kg^{-1} soil)	Chlorophyll content	Panicle No.	Panicle length (cm)	Total No. of grain	100-grain weight (g)
0	21.84b	8.85 c	21.25b	63.34b	2.02b
10	24.37a	13.26a	23.70a	81.19a	2.06a
20	25.54a	10.96b	23.72a	81.25a	2.08a

Different letter(s) (a, b, c) indicates that the differences between data are significant at 5%, The values represent the mean of four replications

Table 4: Differences of significance traits for Zn×S interactions in two typical genotypes at 10 mgZn kg^{-1}

Genotypes	Zn×S	Total filled grain	100-grain weight	Chlorophyll content
Shafagh	0	62.25a	2.28a	26.63a
(Zn efficient)	10 6	42.58b	2.22a	24.25ab
	decrease (%)	31.59	2.63	8.93
IR26	0	58.16ab	2.26a	22.70bc
(Zn inefficient)	10 6	23.91c	1.98b	20.23c
	decrease (%)	58.88	12.38	10.88

Different letter(s) (a, b and c) indicates that the difference between data is significant at 5%. The values represent the mean of four replications

(Table 1 and 3) and the genotypes had significant differences in all of the measured traits except panicle number per plant (Table 1).

The agronomical parameters of the rice plants increased as the Zn concentration in soil was increased. The application of Zn at rate 10 mg Zn kg^{-1} had more additional effect on panicle number (33.2%) than the others (Table 3). At concentrations of 10 mg Zn kg^{-1} soil or higher, the agronomical parameters of the plant were about the same for staple traits, but increase of Zn level reduced panicle number per plant significantly. On this base, concluded that rate of 10 mg Zn kg^{-1} was recommendable for rice plant. Also, increase of Zn level from 0 to 20 mg kg^{-1} elevated the CC about 12 and 17%, for 10 and 20 mg Zn kg^{-1}, respectively (Table 3).

Also, result of salinity treatment on agronomical traits was similar to genotype effect. Salinity treatment (6 dS m^{-1}) caused the most declines for total filled grain (64.2%) among studied traits (Table 2).

Furthermore, interactions of Zn×genotype and interaction of Zn×genotype×salinity were only significant for 100 grain weight. Results indicated that Zn×salinity interaction effects on total filled grains, 100 grain weight and chlorophyll content were varied (Table 1).

Mean comparison (DMRT 5%) of significance traits for Zn×S interactions at the best level of Zn (10 mg Zn kg^{-1}) indicated that decline percentages of these characteristics under salinity treatment were higher in the Zn-inefficient genotypes (Table 4).

Salinity effect on Zn content: Shoots Zn content under salinity treatment and zinc levels were varied in rice genotypes. Zinc concentrations in shoot increased with increasing Zn level in saline and nonsaline conditions in six genotypes (Fig.1). Results showed that salinity treatment induction at booting stage, decreased Zn content at control (0 mg Zn kg^{-1}) in rice genotypes except for IR9764 and IR9884 (Zn efficient). But at 10 mg Zn kg^{-1}, salinity treatment caused significant reduction in zinc content in Hashemi, Domsiah and Kados, (58.4, 28.6 and 70% respectively) and increased slightly in Shafagh, IR9764 and IR9884, (18.4, 31.4 and 11.9 % respectively). The results of salinity treatment at 20 mg Zn kg^{-1} level were also similar to 10 mg Zn kg^{-1} (Fig.1).

The salinity effect on proline content: Salinity effects on proline content at different levels of zinc showed that salinity may elevate the proline content in all rice

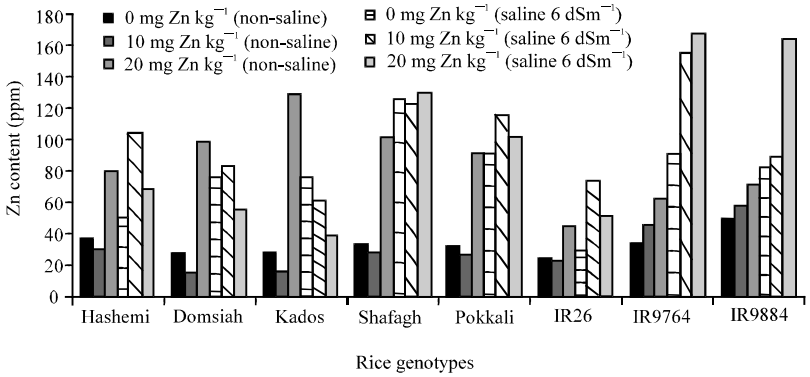

Fig. 1: Zn content in shoot of rice genotypes at booting stage under salinity treatment and different levels of Zinc

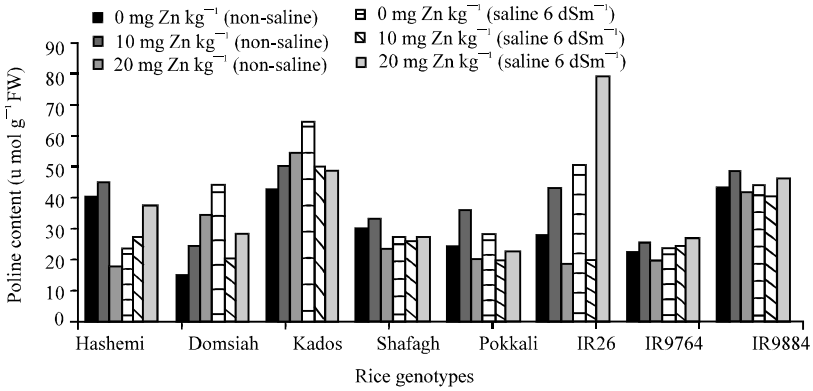

Fig. 2: Proline content in whole plantles of rice genotypes under salinity treatment at different levels of Zinc

genotypes and different levels of zinc (Fig. 2). The result presented indicates that Shafagh, Pokkali, IR9764 and IR9884 had lower fluctuations in proline content after salinity treatment induction. However, in other genotypes, differences in proline content were highly varied in all zinc levels, especially in the IR26 (Fig. 2).

DISCUSSION

No fertile soils tend to have multiple mineral deficiencies and toxicities, so multiple plant tolerances may be required. In saline rice fields, Zn and P deficiencies are more or less universally present (Quijano-Guerta *et al*., 2002). Zinc is not available such as soils, because Zn sorbs or precipitates in unavailable forms (Khoshgoftar *et al*., 2004). Zinc performs various important roles in protecting cells from the damaging reactions caused by ROS. Zinc is required for maintenance of integrity of biomembranes (Marschner, 1995). Under Zn deficient conditions there is a typical increased in plasma membrane permeability in Zn-deficient plants, particularly in the soils affected by salinity. Thus, under these conditions plant growth and yield may decrease (Marschner, 1995).

In present study, salinity treatment decreased some of the main components of rice yield significantly. On the other hand, application of Zn fertilizer partly counteracted negative effects of salinity on plant growth. Accordingly, application of Zn increased salt-tolerance of rice. The results of previous studies have shown that tolerant rice genotypes to Zn deficiency often also have tolerance to salinity (Quijano-Guerta *et al*., 2002; Verma and Neue, 1984). Therefore, it may be found some Iranian rice genotypes with different zinc efficiency under salinity conditions. Study on simple effect of factors, showed that each of them can be more effective than interactions on variations. However, it obtained that genetic variation was suitable relatively, but independent functions of Zn and G caused the non significant interaction between them and accordingly in Zn×G×S interaction, except in 100 grain weight (Table 1). The results of a study in Brazil for zinc uptake efficiency in 10 rice genotypes, showed non significant Zn×G interaction for all of traits, except panicle number (Fageria, 2001). These results demonstrated that zinc and genotype functions were independent.

But Zn×S interaction was not similar with the others. It was significant only for three main parameters of growth and rice yield and for the others were non significant. A survey on effect of zinc in tomato growth under salinity in Turkey conducted that Zn×S interaction was non significant for fresh and dry weight of the plants (Alpaslan *et al*., 1999). Thus zinc and salinity were related together for influence on some of traits. The results of comparison between Zn×S interactions in two typical rice cultivars for the best level of zinc, demonstrated that reduction of total filled grain in Zn-inefficient genotype was almost two folds more than the Zn-efficient (Table 4). Investigation of the mentioned parameters suggests that total filled grain can be used as a criterion for grouping of efficient and inefficient rice genotypes.

Also chlorophyll content had lower reduction in Zn-efficient than to inefficient genotypes. That can be explained by the role of Zn in protein synthesis and also reactive oxygen species are to be expected in Zn-stressed plants, leading to the peroxidation of membrane lipids and, thus, the cooxidation of chlorophyll (Marschner and Cakmak, 1986).

We conducted that zinc accumulation in Zn-efficient genotypes such as Shafagh, IR9764 and IR9884 was higher than Zn-inefficient like IR26 (Fig. 1). This result refers to higher ability of Zn-efficient genotypes in translocation of zinc from root to shoot. But, salinity treatment caused to reduce zinc accumulation in Zn-inefficient genotypes. A study demonstrated that salinity affects not only the bioavailability of soil Zn but also modifies plant functions related to their acquisition and translocation to the leaves (Helal *et al*., 2000).

Proline is well known as an osmoregulatory solute in plants subjected to hyperosmotic stress (Delauney and Verma, 1993). Osmotic Adjustment (OA) is an effective criterion against abiotic stress (salinity) tolerance in many plants, including rice (Datta, 2002). In rice, proline accumulation seems to be symptom of injury rather than an indicator of salinity resistance (Moradi, 2002; Lutts *et al*., 1999). Indeed, salt-resistant cultivars accumulate lower amounts of free proline than salt-sensitive ones (Lutts *et al*., 1999). The results of this study demonstrated two different pattern of proline content in genotypes (Fig. 2). In Zn-efficient genotypes (group I: Hashemi, Domsiah, Kados, IR26) total proline was lower in all Zn levels than the Zn-inefficient genotypes (group II: Shafagh, Pokkali, IR9764, IR9884).

In general, literature review shows 100 grain weight (Fageria, 2001) and total number of filled grain (Mehertre *et al*., 1994) are the most important rice yield components that can be affected by zinc and salinity. Also, the measurement of Chlorophyll Content (CC) is an index for fertilizer management and salt tolerance screening (Wanichananan *et al*., 2003).

On the basis of above characteristics, the experiment showed that the Zn-efficient cultivars of low land rice were tolerant to salinity conditions. The results suggest that zinc has a regulatory or control mechanism on salinity injury, especially in Zn-efficient cultivars. It seems that a complex mechanism of the enzymes function affected by Zn to rule on salinity tolerance.

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