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Metabolic variation in rice cultivars of contrasting salt tolerance and its improvement by zinc in sodic soil

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Abstract: *The severity of Zn deficiency increased with increase in soil exchangeable sodium percentage (ESP) with salt sensitive variety M1-48 scoring 6 at ESP 62 as against only score 3 by salt tolerant variety Pokkali under similar soil conditions. Strikingly, zinc contents were much higher in salt tolerant variety than in salt sensitive one. Zinc application increased zinc concentration in the roots by a factor of 2.85 to 3.87 in Pokkali whereas it rose from 2.37 to 4.35 times in M1-48 depending upon ESP but in the leaves it registered increase of 1.5 to 1.8 times only. In general, the concentrations of reducing sugar were less (about 2.2%) than that of non-reducing (about 3.8%) in both the varieties under normal soil conditions. However, the concentration of reducing sugar doubled (4.2-4.4%) at the highest ESP 62, whereas the concentration of non-reducing sugar though increased (4.1 to 5.1%) but not as vigorously as reducing one. Zinc application reduced the concentration of reducing sugar but not that of non-reducing at similar ESP values. In Pokkali, the concentrations of total sugar increased from 6% at ESP 20 to 9.34% at ESP 62 , whereas it registered enhancements of 5.98 to 8.6% in M1-48 under similar conditions. The nitrate reductase (NR) activity decreased with increase in soil sodicity however, the varietal differences in NR activity were wider under Zn-stress than under conditions of applied zinc with Pokkali registering higher NR activities.Carbonic anhydrase activities were higher in salt tolerant variety. Inhibition in carbonic anhydrase activity amounted to 23 and 45% in salt-sensitive variely M1-48 whereas only 19 and 33% in salt-tolerant variety Pokkali at ESP 41 and 62, respectively. The effects of zinc application at higher soil sodicity were more obvious in salt-sensitive variety than in salt-tolerant one. The findings suggest that the tolerance to Zn stress runs parallel to salt tolerance abilities of rice varieties.*

Key words: *Carbonic anhydrase, Metabolic variation, Nitrate reductase, Salt tolerance, Sodic soil, Zinc nutrition, Zn stress.*

Introduction

Zinc is an essential component of cereal cultivation technology for salt-affected soils (Singh 1982, 1983). The effects of controlled levels of soil factors such as high pH and exchangeable sodium and that of environmental factors such as water regime, temperature, light intensity and its spectral composition on zinc nutrition, growth and yield of rice have been only sporadically studied (Wallace, 1966; Singh and Singh 1999).

In addition to soil and environmental factors, zinc nutrition of rice may also be affected by plant factors, or the effects of soil and environmental factors may greatly be modified by plant factors (Gill, 1992; Muhling and Lauchli, 2002). For instance, species and cultivars have been shown to vary significantly in their abilities to obtain macro and micronutrients including zinc from a given soil (Giordano and Mortvedt, 1974; Agarwala *et al.,* 1978).

In line with these findings, varietal differences in zinc nutrition are now well documented in literature, but are rather poorly understood. Therefore, with a view to understand more about the mechanism of varietal differences in zinc nutrition, the present investigations were carried out on two rice varieties differing greatly in salt tolerance at varying levels of exchangeable sodium in soil. To further enhance the practicalability of the findings, additional treatments were given to explore the possible ameliorative effect of zinc on metabolic drift.

Materials and Methods

The investigations were carried out in pot culture under open green-house conditions. To obtain various exchangeable sodium percentages such as ESP 20, 40 and 60 soils collected from a normal field, were sprayed with $NaHCO_3$ solutions of known strength as per the method described by Joshi and Singh (1975) and subsequently heaped, covered fully by ploythene sheets and incubated as such under moist conditions for a period of one month before they were used for pot culture. The soils were filled in earthen pots of 8kg capacity each lined inside with polythene bag. Soils in each pot were adequately fertilized with NPK fertilizers, irrigated to enable 5cm of water to stagnate and transplanted at the rate of 2seedlings/hill and 3hills/pot. Pokkali, a salt tolerant and M1-48, a salt-sensitive variety constituted the experimental plant materials. Zinc was applied at the rate of 0, 20 or 40kg $ZnSO_4$/ha (on soil weight basis) at the time of transplanting to make it a 3x3x2 factorial experiment with 3 replications. At the time of pot filling soil samples were drawn from each lot and pH values were determined. From the observed pH values the corresponding ESP values were extrapolated (Richards, 1954) which calculated to 20, 41 and 62 respectively and the results have been described in the light of these ESP values. Two weeks after transplanting scores ranging from 1 to 9 on zinc deficiency symptoms as outlined in standard evaluation system for rice were recorded (IRRI, 1980). Score I means growth and tillering nearly normal and score 2 means

Table – 1: Variability in sugar metabolism of rice cultivars of contrasting salt tolerance in sodic soil and its modification by zinc application.

Level of sodicity (ESP)	Genotype of rice	Zn deficiency symptoms (score)			Reducing sugar (%)			Non-reducing sugar (%)			Total sugar (%)		
		Level of $ZnSO_4$(kg/ha)			Level of $ZnSO_4$(kg/ha)			Level of $ZnSO_4$ (kg/ha)			Level of $ZnSO_4$(kg/ha)		
		0	20	40	0	20	40	0	20	40	0	20	40
20	M1-48	2.3	1.3	1.0	2.21	2.05	1.78	3.77	3.90	4.10	5.98	5.95	5.88
	Pokkali	1.3	1.0	1.0	2.18	2.00	1.84	3.91	4.04	4.15	6.09	6.04	5.99
41	M1-48	4.3	2.7	1.7	3.25	2.69	2.12	4.01	4.33	4.65	7.26	6.99	6.78
	Pokkali	2.0	1.0	1.0	3.15	2.65	2.0	4.70	5.04	5.41	7.85	7.69	7.41
62	M1-48	6.3	4.3	3.0	4.45	3.87	3.31	4.15	4.46	4.50	8.60	8.33	7.81
	Pokkali	3.0	2.0	1.3	4.20	3.35	2.50	5.14	5.46	5.80	9.34	8.81	8.30
	LSD(0.05)	0.9	0.7	0.7	0.38	0.32	0.29	0.08	0.08	0.09	0.14	0.13	0.12

Table – 2: Variability in zinc partitioning in rice cultivars of contrasting salt tolerance in sodic soil and its modification by zinc application.

Level of sodicity (ESP)	Genotype of rice	Zinc in leaf (ppm) Level of $ZnSO_4$(kg/ha)			Zinc in root (ppm) Level of $ZnSO_4$(kg/ha)		
		0	20	40	0	20	40
20	M1-48	18.6	23.1	28.8	32.3	36.5	76.7
	Pokkali	20.8	28.1	36.0	38.8	50.3	110.9
41	M1-48	14.9	19.4	23.6	23.4	28.7	68.1
	Pokkali	18.2	24.4	31.6	29.3	42.2	95.2
62	M1-48	11.0	15.2	20.1	14.2	18.8	57.3
	Pokkali	16.1	21.2	25.3	20.3	29.9	78.6
	LSD(0.05)	0.7	0.9	0.9	0.8	1.7	2.8

though growth and tillering normal but basal leaves are slightly discoloured. However, score 3 indicates slight stunting, decreased tillering and some basal leaves are brown or yellow, whereas score 5 means growth and tillering severely retarded and about half of all leaves are brown or yellow. Growth and tillering ceased, most leaves are brown or yellow in plants having score 7 and score 9 means that almost plant is dead or dying. Zinc content in plant tissues and reducing and non–reducing sugars in leaves were estimated according to Yoshida *et al*. (1976). Nitrate reductase activities in leaves were determined as per methods developed by Hageman and Hucklesby (1971) whereas carbonic anhydrase activities were determined by colorimetric method of Wilber and Anderson (1948). Data were analyzed statistically (Li, 1968).

Results and Discussion

Genotypic differences in zinc nutrition between the two rice varieties in response to a range of soil sodicity were quite apparent in accordance with their reputed genetic variation in salt tolerance (Table1). The susceptible variety M1-48 developed quite severe Zn-deficiency symptoms (score 6, growth and tillering very severely retarded) at ESP 62, whereas Pokkali which is known for its greater salt tolerance (IRRI 1982) suffered only minor setback (score 3) in developing Zn-deficiency symptoms. Zinc concentration accordingly dropped in plant tissues with increase in soil sodicity. Varieties differed much from each other with respect to zinc concentration under conditions of sodicity with tolerant accumulating more than the sensitive one (Table 2). Zinc deficiency symptoms were found to be related to Zn content in leaves rather than those in roots. Upon zinc application the zinc concentration, in general, increased about 4-fold in the roots but only 1.5 to 1.8 times in the leaves. Thus, a large proportion of total zinc absorbed was retained by roots themselves. The possible causes of inhibition of Zn translocation from root to shoot have been discussed by Wallace (1966), Taylor *et al.* (1989) and Singh and Singh (1999). These inhibitions include zinc compartmentation within roots itself (Turner and Marshall, 1971,1972) as well as binding of Zn with certain peptides (Taylor *et al.,* 1989) which may lead, among other factors, to varietal differences of the kind reported herein (Brookes *et al.,* 1981). Increase in soil sodicity resulting in concomitant decline in zinc status particularly in leaves, caused profound changes in plant metabolism (Dwivedi and Randhawa, 1974, Sharma *et al.,* 1981). In general, total sugar increased with increase in soil exchangeable sodium (Table 1) and these enhancements were shared by both the reducing as well as non-reducing sugars. Interestingly, zinc application reduced the concentration of reducing sugar whereas

Table – 3: Variability in nitrate reductase and carbonic anhydrase activities in rice cultivars of contrasting salt tolerance in sodic soil and its modification by zinc application.

Level of sodicity (ESP)	Genotype of rice	Nitrate reductase activity (µg NO_2/ g dry wt./hr) Level of $ZnSO_4$(kg/ha)			Carbonic anhydrase activity (µg CO_2 / 100mg dry wt./10min.) Level of $ZnSO_4$(kg/ha)		
		0	**20**	**40**	**0**	**20**	**40**
20	M1-48	58.3	61.0	62.3	336	368	385
	Pokkali	62.3	64.3	64.7	580	602	617
	M1-48	50.7	56.7	58.3	258	302	342
41	Pokkali	55.0	59.3	60.3	471	504	525
	M1-48	37.7	43.0	50.3	185	272	285
62	Pokkali	43.3	46.7	52.3	389	437	455
	LSD(0.05)	1.2	1.4	1.4	22	24	26

concentrations of non-reducing sugar increased. The concentrations of sugar at any level of soil sodicity were more in Pokkali than in M1-48. Our observations are in sharp contrast to those of Sharma *et al* (1981) who reported a marked accumulation of reducing sugars in response to Zn-deficiency in *Citrullus* plant. Decrease in nitrate reductase activity on account of increased soil sodicity is another fascinating example of metabolic effects of Zn-deficiency (Table 3).The sensitivity of nitrate reductase activity to zinc deficiency may be due to a variety of factors including a possible inactivation of the enzyme. Zinc application restored nitrate reductase activity to a large extent under alkaline conditions, however, no genotypic variability could be detected. Thus, zinc application at the rate of 40kg $ZnSO_4$/ha restored NR activity very effectively such that reductions amounted to only 1% at ESP 41 and 14-15% at ESP 62. The restoration in the activity of this enzyme upon Zn–application suggests the possible involvement of zinc in nitrogen and protein metabolism. The changes in the activity of carbonic anhydrase are not unique in itself on account of Zn-deficiency caused by high sodicity since carbonic anhydrase is a zinc-metallo-enzyme (Srivastava and Rathore, 1985). Therefore, Zn-deficiency, as expected, caused dramatic decline in its activity. Upon zinc application its activity, was also restored to a large extent. Decrease in carbonic anhydrase activity has also been noted in species other than rice by other workers (Sharma *et al.,* 1981; Srivastava and Rathore, 1985). Relatively less reduction in carbonic anhydrase activity in Pokkali appears to be regulated, among other things, through zinc content of leaves and probably, it is the variation in zinc nutrition between these two varieties which is responsible for observed varietal differences in salt tolerance. On the basis of the present findings, it is hypothesized that increase in sodicity causes decrease in zinc status of leaves which, in turn, results in a series of quantitative and qualitative metabolic changes (Ponnamperuma, 1984; Ashraf *et al.,* 1991; Gehlot *et al.,* 2003) leading to genotypic variation in growth and survival of rice under conditions of abiotic stress, i.e. salt stress and /or Zn-stress.

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