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Zinc/phosphorous ratio in shoot as an index of evaluating rice salt tolerance

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Rice genotypes of varying salt tolerance [tolerant (T), semi-tolerant (ST) and sensitive (S)] were evaluated in field lysimeters that contained saline soil of EC_e 4 and 8 $mS\ cm^{-1}$ and alkali soil of pH 9.5 and 9.8 in Karnal, North India. Tolerant rice genotypes accumulated lesser Na^+ and higher K^+ , which led to lower Na/K ratios in shoot; and they showed non-significant reduction in Zn content even at EC_e 8 whereas they were significantly reduced in ST and S groups even at EC_e 4 $mS\ cm^{-1}$. Alkalinity had no significant effect on Zn content of any group. There was significant increase in shoot P with increasing stress in T and ST groups. Zn/P ratio in shoot reduced with salinity stress in all the tolerance groups but there were no marked effects of alkalinity. A Na/K ratio of <1.1 at high salinity and <0.8 at high alkalinity in the shoots served to distinguish the tolerant rice genotypes from the sensitive. The T genotypes had a shoot Zn/P ratio of >0.03 whereas the sensitive had <0.02 under salinity stress. Results suggested that in addition to Na/K ratio, shoot Zn/P ratios can be used as additional criteria for identifying rice genotypes with higher tolerance under salinity (but not alkalinity) stress.

Key words: Potassium, salt stress, salinity, alkalinity, sodium.

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food of south and Southeast Asia where soil salinity and alkalinity (sodicity) seriously reduces productivity in about 100 million hectare (Hossain and Fischer, 1995; Mavi et al., 2012). This requires a major focus on improving the productivity and sustainability of rice based farming systems so as to ensure livelihood and nutritional security. Selection/breeding of salt tolerant rice genotypes has been carried out for over three decades (Flowers, 2004). One of the most useful approaches that have been used to identify salt resistant genotypes is a low Na/K ratio in the shoots (Yeo and Flowers, 1982; Bal et al., 1986). There was a significant negative correlation between leaf Na^+

and K/Na ratio in varieties under salt stress (Haq et al., 2009). It was further observed that in the case of rice genotypes grown under saline stress conditions, the K content in shoot was significantly and positively correlated with grain yield per plant. High K uptake plays an important role in imparting reproductive stage salt tolerance in rice and improving grain yield under stress. (Mishra, 1994).

High soil phosphate levels are one of the most common causes responsible for zinc deficiency in crops (Cakmak, 2000; Marschner, 1993) which is one of the most widespread micronutritional disorders of food crops the world over (Lopes, 1980; Alloway, 2009). It is more common in wetland rice than in dryland crops (Castro, 1977; Randhawa et al., 1978) as the concentration of zinc decreases upon flooding. Zn deficiency has thus been identified as one of the most important nutritional stresses limiting rice production in Asia (Cakmak et al.,

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2001; Quijano-Guerta et al., 2002; Luo et al., 2010; Rehman et al., 2012). The growth of plants growing in salt affected soils besides being adversely affected due to direct osmotic effect of salts is also affected due to nutritional imbalance caused by reduced availability of zinc and phosphorus in saline soils. In alkali (sodic) soils, due to high pH, low organic matter, presence of calcium carbonate, high soluble P, toxic concentrations of carbonate and bicarbonate, the available zinc is low due to which plants show zinc deficiency leading to yield reduction.

Plant biologists have relied so far on the vigor of the cultivar at vegetative stage, reproductive stage tolerance, high grain yield and low transport of sodium into shoots as criteria for selecting varieties tolerant to salt stress. Additional favorable traits contributing to tolerance like the ability to take up higher amount of phosphorus and zinc (Aslam and Qureshi, 1989) have not been considered so far. Previous studies on zinc and phosphorous uptake of rice genotypes have been conducted under controlled *in vitro* conditions using single salts, mostly NaCl and also screening only a few rice varieties. However salinity is a complex stress- in nature the soil solution is a diverse mixture of various cations like Na^+ and Ca^+ and anions like Cl^- and SO_4^{2-} . Secondly, the evaluation of tolerance to salinity and sodicity stresses has been conducted separately by different workers using different sets of genotypes for the two types of stresses. If conducted at the same place, then these were done with a limited number of genotypes using mostly one or two representatives of each category *viz.*, tolerant, semi-tolerant and sensitive and also sometimes in different years/growing seasons. This makes broad generalizations of the effects of salinity and alkalinity tolerance on zinc and P uptake in relation to tolerance difficult. Therefore we conducted a comprehensive study, simultaneously screening a large number of varieties: 8 tolerant, 8 semi-tolerant and 3 sensitive rice genotypes (total no.19) in both saline and alkali (sodic) soils in lysimeters installed adjacent to rice fields. We analyzed the P and Zn uptake in plants to see the contribution of better nutrient uptake if any to tolerance as assessed by grain yield (Surekha Rao et al., 2008), Na and K uptake under salinity and alkalinity stresses and test the hypothesis that Zn/P ratio in plants contributes to salinity and alkalinity tolerance.

MATERIALS AND METHODS

Experiment set-up

Nineteen (19) rice genotypes representing a range of tolerance to salt response were selected for this study conducted at the Central Soil Salinity Research Institute (CSSRI), Karnal, Haryana in northern India. This study area is typical of semi-arid sub-tropical India with climatic conditions of hot and dry summers and cold

winters. Rice genotypes evaluated in this study ranged from traditional, tall land races to bred dwarfs and are cultivated in different agro-ecological regions of the Indian sub-continent. Of the 25 rice genotypes initially screened in lysimeters at salinity of EC_e 4 and 8 mS cm^{-1} (average root zone salinity) and alkalinity of pH_2 9.5 and 9.8, six yielded a mixed response. The rest of the 19 genotypes could be characterized as tolerant, semi-tolerant and sensitive depending on their absolute yield and relative yield reduction under both types of stresses (Surekha Rao et al., 2008). The origin and parentage of the genotypes and other plant characteristics are given in Table 1. Total volume of each lysimeter was 27 m^3 each was filled up with 4 t of normal soil. One set was salinised by adding 8.3 kg NaCl, 1.5 kg Na_2SO_4 and 2.2 kg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and another set was alkalized by adding 40 kg NaHCO_3 / lysimeter. The soils were repeatedly wetted and dried for two seasons to ensure uniform equilibration with added salts. Two levels each of soil salinity, (EC_e 4 and 8 mS cm^{-1}) and alkalinity (pH_2 9.5 and 9.8) as measured by the average electrical conductivity of soil saturation extract (EC_e) and pH of soil:water suspension in 1/2 ratio (w/v) during the entire growth period of rice were achieved. Normal soil (pH_2 7.3, EC_e 1.2 mS cm^{-1}) was used as a control treatment. Measurements on pH and EC_e were made using portable pH and EC meters four times during growth period of each crop. Cation exchange capacity (CEC) was determined by Bower's ammonium acetate method and organic carbon by Walkley-Black wet oxidation procedure. Total N was determined by Kjeldahl method and available N by alkaline permanganate oxidation and distillation; available P by spectrophotometry using Olsen extractable (0.5 M NaHCO_3 , pH 8.5) method and available K (1M NH_4COOH -extractable) using flame photometry following procedures as described in Hesse (1971). The soils were sandy loam in texture, low in available N, medium to high in P and high in K. The salient soil physicochemical and fertility properties are listed in Table 2. Thirty to thirty-five day old seedlings of each rice genotype were transplanted in 1.5 m long rows in three replications using randomized block design layout. Two seedlings per hill were transplanted 15 cm apart in each row whereas distance between the two rows was kept 20 cm. Phosphorus as single super phosphate at $40 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ and zinc as $20 \text{ kg zinc sulphate ha}^{-1}$ were incorporated into the surface (0 to 15 cm) soil prior to puddling. Nitrogen was applied by broadcasting urea at 20 kg ha^{-1} in three splits of 40 kg each at transplanting, maximum tillering and flowering stage. Insecticidal sprays were applied as plant protection measures whenever needed. Ponding with 7 to 8 cm deep water was maintained throughout the experimental period, from transplanting until completion of grain filling.

Plant chemical analysis

The shoot samples at maturity were collected in triplicate, digested in di-acid mixture (nitric acid and perchloric acid in, 3/1 ratio) and analyzed for Na and K using a flame photometer (EEL, Corning, UK) and expressed on percent dry weight basis. Phosphorus concentration in the digest was estimated by phospho-vanado-molybdate yellow color method on a spectrophotometer (Shimadzu U.V- 2100S). Zinc was determined with an atomic absorption spectrophotometer (GBC).

Statistical analysis

The Na^+ , K^+ and Na/K ratio, Zn, P and Zn /P ratio data of all the 19 genotypes were subjected to analysis of variance (ANOVA) using SPSS package to compute genotypic (G) and environmental effects (E) and G x E interactions across all 5 environments. ANOVA was

Table 1. Parentage, plant characteristics and ecological origin of the rice genotypes screened for tolerance to salinity and sodicity

Genotype	Parentage/characteristics	Plant type	Grain shape	Origin/source
CSR (Damodar)	Land race	Tall	Bold	Saline marshy lands, Sunderbans (W. Bengal)
CSR 10	M40-431-24-114/Jaya	Dwarf	Short bold	CSSRI, Karnal
CSR 11	M40-431-24-114/Bas 370	Dwarf	Short bold	-do-
CSR 13	CSR1/Bas370//CSR5	Semi dwarf	Long Slender	-do-
CSR 18	RPA 5829/CSR5	Semi dwarf	Long Slender	-do-
CSR 21	IR5567-33-2/ IR4630-22-2-5-1-3	Semi dwarf	Medium Slender	CSSRI, Karnal; Anther culture derivative (IRRI)
CSR 22	IR64/IR4630-22-2-5-1-3/ IR9764-45-2-2	Medium Semi dwarf	Medium Slender	CSSRI, Karnal
CSR 27	N.Bokra/IR5657-33-2	Semi dwarf	Long slender	-do-
CSR 29	IR14632-22-3/ IR19799-17-3-1-1	Semi dwarf	Long slender	-do-
CSR 30	Bhura Ratta 4-10/Pak Basmati	Tall	Long slender	-do-
Pokkali	Land race	Tall	Short bold	Kerala
Panvel – 1	IR8/Bhura Ratta 4-10	Semi tall	Short bold	Maharashtra
CO 43	Dasal/IR20	Semi dwarf	Medium Slender	Tamil Nadu
Pusa Basmati 1	Pusa 167/Karnal local	Semi dwarf	Long slender	IARI, Delhi
M1-48	Land race	Semi tall	Short bold	Philippines
Bas 370	Pure line selection	Tall	Long slender	Haryana
IR 36	IR1561-228-1-2/ IR1737//CR94-13	Semi dwarf	Long slender	IRRI, Philippines
Jaya	T(N)1/7141	Semi dwarf	Long bold	DRR, Hyderabad
BR-4-10	Land race(Bhura Ratta 4-10)	Tall	Short bold	Maharashtra

Table 2. Salient physico-chemical and fertility properties of the experimental soils.

Property	Normal	Saline-1	Saline-2	Alkali-1	Alkali-2
pH ₂ (1:2)	7.3	8.2	8.7	9.5	9.8
EC _e (mS cm ⁻¹)	1.5	4.2 ± 0.7	8.2 ± 1.7	1.1 ± 0.1	1.3 ± 0.2
CEC (c mol kg ⁻¹)	10.1	11.6	12.4	12.0	13.1
Organic carbon (g kg ⁻¹)	4.6	4.8	5.0	4.4	4.4
Total N (g kg ⁻¹)	0.56	0.42	0.43	0.48	0.50
Av. P (Kg ha ⁻¹)	1.2	9.0	17.0	12.0	19.4
Av. K (Kg ha ⁻¹)	241	179	202	200	225
DTPA-Zn (mg kg ⁻¹)	1.5	1.1	1.2	1.2	1.1

also done and t-test used to detect significant differences and identify genotypes that are specifically tolerant to high salinity (EC_e 8 mS cm⁻¹) or high alkalinity (pH₂ 9.8) or both the stresses having high Zn / P ratio.

RESULTS

The rice genotypes used in this study belonged to traditional land races (tall) as well as those genotypes (medium and dwarf) bred for high yield and tolerance to salinity and alkalinity. The effects of salinity and alkalinity on Na, K and Na/K ratio; Zn, P and Zn/P ratio of shoots at maturity in all the three tolerance groups were analyzed

by ANOVA and the F values were found to be highly significant ($p < 0.0001$) for the genotypic differences and the stress environments (data not shown).

Na and K content

The accumulation of Na⁺ at maturity increased significantly at higher levels of salinity EC_e 8.0 mS cm⁻¹ and higher levels of alkalinity pH₂ 9.8 over normal in both the tolerant and sensitive genotypes (Figure 1). Shoot Na content of tolerant (T) genotypes was 0.82 and 0.77%, of ST genotypes was 0.87 and 0.71% and of S genotypes was 1.16 and 1.50% at EC_e 8 and pH₂ 9.8, respectively.

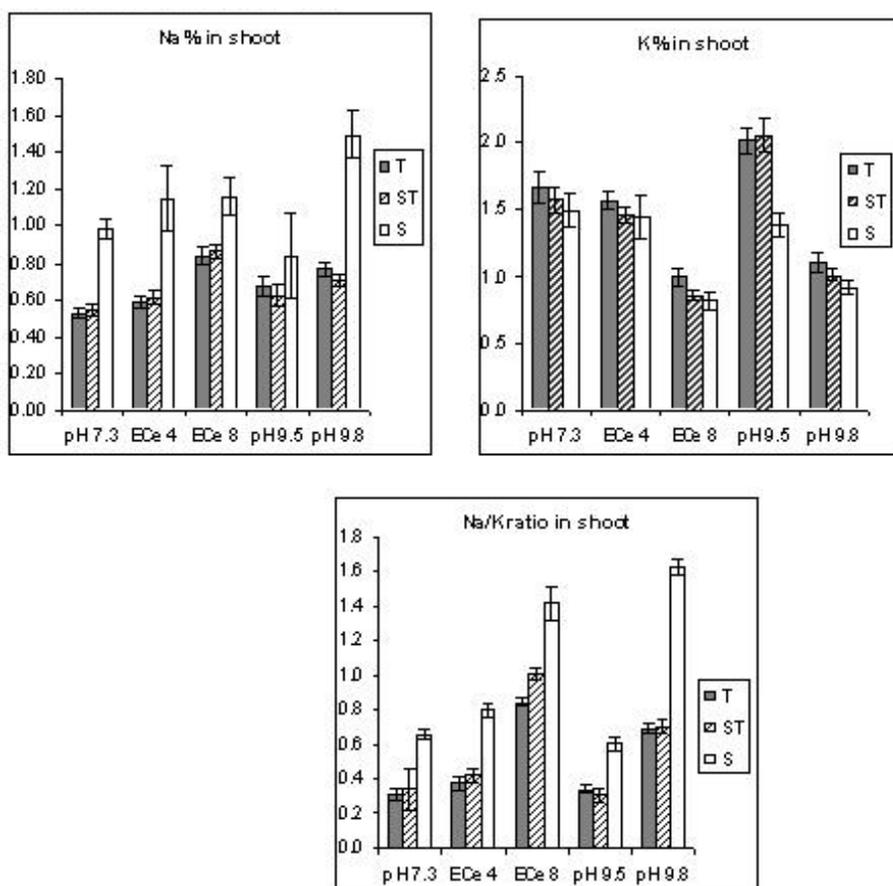


Figure 1. Effect of soil salinity (EC_e $mS\ cm^{-1}$) and alkalinity (pH_2) on Na and K concentration in rice shoots of tolerant (T), semi-tolerant, (ST) and sensitive (S) genotypic groups. Bars indicate SEM.

The average percent increase in the Na content in the shoot was 57.2 and 46.9% in T genotypes, 25.6 and 18.0% in ST genotypes and 18.1 and 51.9% in S genotypes at EC_e 8 and pH_2 9.8 respectively over normal soil. There was a marked and statistically significant decrease in the K content of the shoots at higher level of salinity EC_e 8.0 $mS\ cm^{-1}$ and higher level of alkalinity pH_2 9.8 over normal in all the genotypic classes (Figure 1). The shoot K content at EC_e 8 and pH_2 9.8 of T genotypes was 5.3 and 4.2% respectively, of ST genotypes was 4.9 and 4.3%, of S genotypes was 4.1 and 4.3%. The average percent decrease in K content in shoot was 65.2 and 31.7% in T genotypes, 77.9 and 88.5% in ST genotypes and 40.1 and 36.2% in S genotypes at EC_e 8 and pH_2 9.8 respectively (Figure 1) over normal soil.

Na/K ratio

The Na/K ratio in shoot of all the tolerance classes showed a statistically significant increase at higher level

of salinity EC_e 8.0 $mS\ cm^{-1}$ and higher level of alkalinity pH_2 9.8 over normal in all the tolerance classes (Figure 1). The tolerant group accumulated lesser amount of Na^+ and higher K^+ in the shoot at maturity in comparison to sensitive leading to lower Na/ K ratios. At EC_e 8 and pH_2 9.8 the shoot Na/K ratio of T genotypes was 0.824 ± 0.027 and 0.694 ± 0.028 respectively, of ST genotypes was 1.007 ± 0.035 and 0.702 ± 0.038 , and of S genotypes was 1.415 ± 0.096 and 1.623 ± 0.045 respectively (Figure 1). The average percent increase in shoot Na/K ratio was 162.3 and 120.9% in T genotypes, of 130 and 60.4% in ST genotypes and of 114.7% and 146.2% in S genotypes at EC_e 8 and pH_2 9.8 respectively (Figure 1) over normal soil.

Zinc and phosphorus content

The tolerant rice showed statistically non-significant reduction in Zn content of the shoot even at high salinity of EC_e 8 $mS\ cm^{-1}$ whereas it was reduced in the semi-

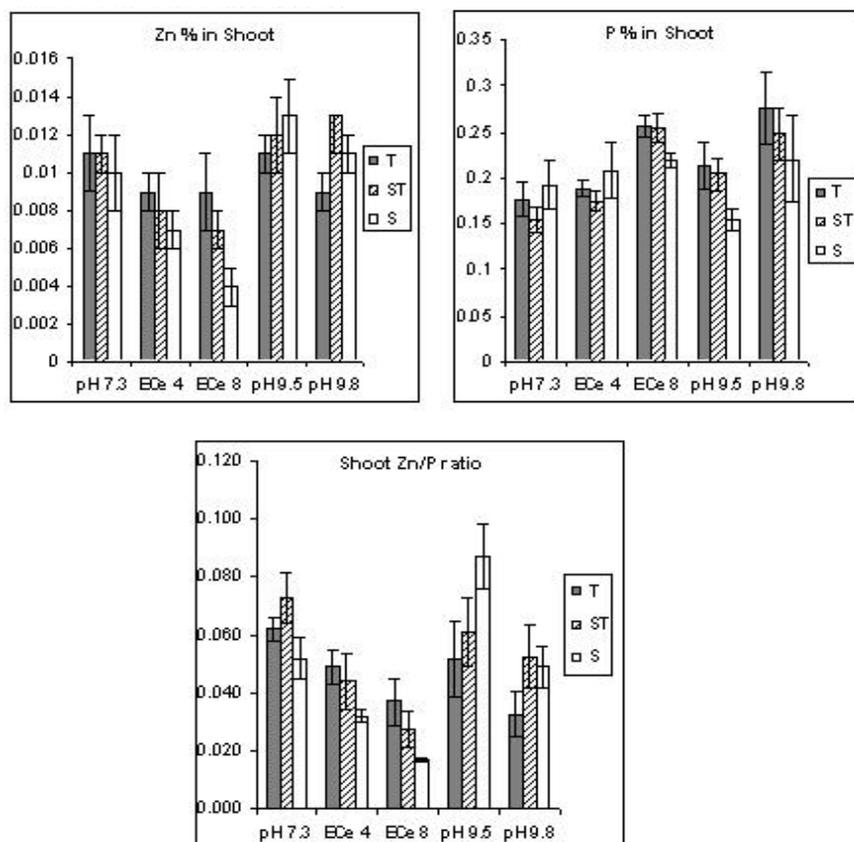


Figure 2. Effect of soil salinity (EC_e $mS\ cm^{-1}$) and alkalinity (pH_2) on Zn and P concentration in rice shoots of tolerant (T), semi-tolerant (ST) and sensitive (S) genotypic groups. Bars indicate SEM.

tolerant and sensitive groups even at EC_e $4\ mS\ cm^{-1}$. Alkalinity had no significant effect on Zn content of any tolerance group. The shoot Zn content at EC_e $8\ mS\ cm^{-1}$ and pH_2 9.8 of T genotypes was similar, 0.009%. In ST genotypes, it was 0.007 and 0.013%, of S genotypes was 0.004 and 0.011% (Figure 2). The average reduction in T genotypes was 18.2% (NS) at EC_e 8 and pH_2 9.8 (NS); decreased by 31.2% at EC_e $8\ mS\ cm^{-1}$ and increased by 18.2% (NS) at pH_2 9.8 in ST genotypes; it decreased by 60.0% at EC_e $8\ mS\ cm^{-1}$ and increased by 10.0% (NS) at pH_2 9.8 in S genotypes (Figure 2) over normal soil. The P content in shoot of the T and ST genotypes showed a statistically significant increase at higher levels of salinity EC_e 8 and alkalinity pH_2 9.8 whereas S genotypes showed no effect in P content. The shoot P content at EC_e 8 and pH_2 9.8 of T genotypes was 0.256 and 0.275%, respectively, of ST genotypes was 0.25 and 0.247%, and of S genotypes was 0.220 and 0.220% (Figure 2). The average increase in shoot P content in T genotypes was 44.6 and 55.9% at EC_e 8 and pH_2 9.8 respectively, of 63.9 and of 53.4% in ST genotypes and increase of 14.6% (NS) in S genotypes at EC_e 8 and pH_2

9.8 respectively (Figure 2) over normal soil.

Zn/P ratio

Shoot Zn/P ratio reduced with salinity stress even at EC_e $4\ mS\ cm^{-1}$ in all the tolerance groups but there was no marked effects of alkalinity stress (Figure 2). At EC_e 8 and pH_2 9.8, the shoot Zn/P ratio of T genotypes was 0.037 ± 0.008 and 0.033 ± 0.008 respectively, of ST genotypes was 0.028 ± 0.006 and 0.053 ± 0.011 and of S genotypes was 0.017 ± 0.001 and 0.049 ± 0.007 (Figure 2). The average reduction percentage was 40.5 and 47.5% in T genotypes at EC_e 8 and pH_2 9.8 respectively, reduction of 62.3 and 27.9% (NS) in ST genotypes and reduction of 67.4 and 5.9% (NS) in S genotypes at EC_e 8 and pH_2 9.8 respectively (Figure 2) over normal soil.

DISCUSSION

Screening rice germplasms to locate salt tolerant genes

for use in improving the currently grown varieties is of continuous importance to plant biotechnologists (Flowers, 2004). Rice is considered to be sensitive to salinity and tolerant to alkalinity and some traditional salt tolerant varieties can withstand high pH of up to 10.0 under irrigated conditions (Mishra and Bhattacharya, 1980). The grain yield of many varieties is reduced by half at an electrical conductivity (EC_e) of 6 mS cm^{-1} (Maas and Hoffman, 1977), equivalent and osmotic potential of -0.23 Mpa or 50 Mmol NaCl . Therefore the higher alkalinity level of pH 9.8 and salinity level of 8.0 mS cm^{-1} used in this present experiment were realistic enough to differentiate the responses of the tolerant, semi-tolerant and sensitive genotypes of rice.

In this present study, the Na accumulation was higher in the shoots of sensitive genotypes as compared to tolerant whereas K content showed the reverse trend. The low accumulation of Na and high K content in the shoots imparted tolerance to salinity which is in accordance with Yeo and Flowers (1982, 1993) who showed that significant variability was seen in the degree of tolerance due to differential accumulation of ions within different plant parts. This reflected in terms of Na/K ratio which was also positively correlated with level of salinity and alkalinity tolerance. Na/K ratio in the shoot was lower in T genotypes as compared to S genotypes at EC_e 8 and pH_2 9.8 which supported the views of Bal et al. (1986), Qadar (1988, 1995), Mishra (1994), Yasin et al. (2002), Kanawapee et. al. (2012) that the tolerant genotypes accumulated low Na and high K content and have low Na:K ratio than the sensitive genotypes.

The overall results show that the Na/K ratios of the tolerant and sensitive genotypes fell in distinct classes. Taking the higher stress levels which produced significantly adverse effects on rice (EC_e 8 mS cm^{-1} or pH_2 9.8) tolerant varieties had Na/K ratio <0.85 under salinity and <0.70 under alkalinity whereas sensitive genotypes had Na/K ratio >1.4 under salinity and >1.6 under alkalinity. Factoring in the highest Na/K ratios observed in semi-tolerant genotypes (that fall in the intermediate category), a Na/K ratio of <1.1 at high salinity and <0.8 at high alkalinity in the rice shoots served to distinguish the tolerant genotypes from the sensitive ones.

The growth of plants growing in salt affected soils besides being adversely affected due to direct osmotic effect of salts is also affected due to nutritional imbalance caused by reduced availability of nutrients like zinc and phosphorus at higher salinity and zinc at high alkalinity (Castro, 1977; Randhawa et al., 1978). Salinity adversely affected zinc uptake in all the tolerance groups whereas there was no marked effect of alkalinity stress on the zinc concentration in shoots. The content of phosphorus in the shoots of T and ST genotypes increased at higher levels of salinity (EC_e 8) and alkalinity (pH_2 9.8). Higher content of Zn was observed in the shoots of tolerant genotypes

than in sensitive ones at higher levels of salinity stress as a result Zn/P ratio was higher in tolerant genotypes which could thus serve as an additional indicator of salt tolerance. These results are in accordance with Naeem et.al. (1998) who found that external Zn/P ratio seems to be related to the salinity tolerance of rice. Their study in solution culture on two rice lines of varying salinity tolerance in the presence and absence of NaCl (70 mol m^{-3} NaCl) salinity showed that a higher Zn concentration was required to obtain better yield under saline conditions as compared to that in the normal nutrient solution. There was an inverse relationship between moderately high Zn concentration in the external medium and the concentration of Na, Cl, P and Ca and a positive relationship with K and Zn concentrations. The markedly higher Zn/P ratio under salinity stress in tolerant (0.037) and semi-tolerant groups (0.028) in our study as compared to sensitive groups (0.017) suggests that in addition to Na/K ratio, Zn/P ratios can be used as an additional criterion for identifying rice with higher tolerance and better nutrient uptake under salinity stress but not under alkalinity stress. The tolerant genotypes had a Zn/P ratio of >0.03 in the shoots whereas the sensitive genotypes had a Zn/P ratio <0.02 ; these limits served to distinguish the tolerant genotypes from the sensitive. All the tolerance groups were adversely affected at higher levels of salinity stress at EC_e 8 mS cm^{-1} as the ionic effects on plant growth were overwhelming and particularly so on the sensitive varieties (Surekha Rao et al., 2008) that led to low uptake of both Zn and P that ultimately led to lower Zn/P ratios.

Conclusion

The Zn/P ratios of the salinity tolerant and sensitive genotypes fell in distinct classes but not of the alkalinity tolerant genotypes. Considering the highest ratios in semi-tolerant genotypes that fell in the intermediate category and taking the response at higher salinity level, EC_e 8 mS cm^{-1} into consideration, the tolerant genotypes had a Zn/P ratio of >0.03 in the shoots whereas the sensitive genotypes had a Zn/P ratio <0.02 ; these limits served to distinguish the tolerant genotypes from the sensitive. The markedly higher Zn/P ratio in tolerant and semi-tolerant groups as compared to sensitive groups suggests that in addition to Na/K ratio, Zn/P ratios can be used as an additional criterion for identifying rice genotypes with higher tolerance and better nutrient uptake under salinity stress but not under alkalinity stress.

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