Journal of Stress Physiology & Biochemistry, Vol. 9 No. 1 2013, pp. 54-65 ISSN 1997-0838

ORIGINAL ARTICLE

Physiological Response to Salinity and Alkalinity of Rice Genotypes of Varying Salt Tolerance Grown in Field Lysimeters

P. Surekha Rao[1], B. Mishra[1], S.R. Gupta[2], A. Rathore[3]

[1] *Central Soil Salinity Research Institute, Karnal-132 001, Haryana, India.*
[2] *Kurukshetra University, Kurukshetra-136119, Haryana, India*
[3] *IISS, Nabibagh, Bhopal-462.38, Madhya Pradesh, India*

Phone 91-755-2625547; 91-9329524374
*E-Mail: **psurekha_rao@rediffmail.com**

Received September 19, 2012

Soil salinity and alkalinity seriously threaten rice production in south Asia. Improving screening methodologies to identify sources of tolerance for improved breeding for salt tolerant rice is of continuing importance. Rice genotypes of varying salt tolerance, such as tolerant (T), semi-tolerant (ST), and sensitive (S), were grown in field lysimeters in saline soil of EC_e 4 and 8 mS cm^{-1} and alkali soil of pH 9.5 and 9.8 in North India and analyzed for chlorophyll (Chl), sugar, starch and proline in leaves. Chlorophyll a and b decreased due to salinity in all the tolerance groups. However, Chl a was not much affected but chl b increased with alkalinity. Under high stress both at EC_e 8 and pH 9.8 Chl a and b were more in tolerant than in sensitive genotypes. The ratio of Chl a/b was similar in T, ST and S genotypes under salinity stress. Sugar accumulation was higher in T compared to S under normal conditions but under salinity or alkalinity stress the differences were not significant. Leaf starch was highest in T, intermediate in ST and lowest in S genotypes in normal as well as under salinity and alkalinity stress. There was decrease in starch with salinity and alkalinity stress only in T group but not in ST and S group. Proline increased significantly in all the tolerance groups even at low salinity of EC_e 4 mS cm^{-1} or pH 9.5. The salt tolerant genotypes of rice maintained higher levels of Chl a and b, starch and proline under high salinity and alkalinity stress and are the robust criteria for tolerating high salinity and alkalinity.

Key words: Chlorophyll, Osmolytes, Proline, Salt stress, Starch, Sugar

ORIGINAL ARTICLE

Physiological Response to Salinity and Alkalinity of Rice Genotypes of Varying Salt Tolerance Grown in Field Lysimeters

P. Surekha Rao[1], B. Mishra[1], S.R. Gupta[2], A. Rathore[3]

[1] *Central Soil Salinity Research Institute, Karnal-132 001, Haryana, India.*
[2] *Kurukshetra University, Kurukshetra-136119, Haryana, India*
[3] *IISS, Nabibagh, Bhopal-462.38, Madhya Pradesh, India*

Phone 91-755-2625547; 91-9329524374
*E-Mail: **psurekha_rao@rediffmail.com**

Received September 19, 2012

Soil salinity and alkalinity seriously threaten rice production in south Asia. Improving screening methodologies to identify sources of tolerance for improved breeding for salt tolerant rice is of continuing importance. Rice genotypes of varying salt tolerance, such as tolerant (T), semi-tolerant (ST), and sensitive (S), were grown in field lysimeters in saline soil of EC_e 4 and 8 mS cm^{-1} and alkali soil of pH 9.5 and 9.8 in North India and analyzed for chlorophyll (Chl), sugar, starch and proline in leaves. Chlorophyll a and b decreased due to salinity in all the tolerance groups. However, Chl a was not much affected but chl b increased with alkalinity. Under high stress both at EC_e 8 and pH 9.8 Chl a and b were more in tolerant than in sensitive genotypes. The ratio of Chl a/b was similar in T, ST and S genotypes under salinity stress. Sugar accumulation was higher in T compared to S under normal conditions but under salinity or alkalinity stress the differences were not significant. Leaf starch was highest in T, intermediate in ST and lowest in S genotypes in normal as well as under salinity and alkalinity stress. There was decrease in starch with salinity and alkalinity stress only in T group but not in ST and S group. Proline increased significantly in all the tolerance groups even at low salinity of EC_e 4 mS cm^{-1} or pH 9.5. The salt tolerant genotypes of rice maintained higher levels of Chl a and b, starch and proline under high salinity and alkalinity stress and are the robust criteria for tolerating high salinity and alkalinity.

Key words: Chlorophyll, Osmolytes, Proline, Salt stress, Starch, Sugar

Soil degradation due to salinity and alkalinity is a serious environmental problem of global significance, affecting the livelihood and nutritional security in nearly 100 million ha in south and southeast Asia including about 8.4 m ha in India (Tyagi and Minhas, 1998). Rice (Oryza sativa L.) is the staple food of this region and major efforts are underway for improving the rice based farming systems (Hossain and Fischer, 1995; van Nguyen and Ferrero, 2006) to meet the challenges posed by various biotic and abiotic stresses and climate change. Selection/breeding of salt tolerant genotypes has been carried out for over 3 decades (Flowers, 2004) and various screening methodologies used (Flowers and Yeo, 1981; Qadar, 1988; Kuchanur et al, 2006) to screen out tolerant varieties. There is a need to determine the underlying biochemical mechanisms of salinity tolerance so as to provide plant breeders to use these biochemical characteristics as selection criteria for salt tolerance for individual species rather than generalized for all species (Ashraf and Harris, 2004). Physiological responses are the most sensitive indices for screening and knowledge of the genetic variability for related traits and their relationship to yield performance in field are important.

Salt accumulation in leaf reduces photosynthesis and growth (Sudhir and Murthy, 2004), decrease in chlorophyll (Chl) content is a commonly reported phenomenon. But many studies showed that Chl content in the leaves of tolerant rice varieties were maintained better than in the sensitive ones (Khan and Abdullah, 2003; Cha-Um et al, 2009) while some others showed that total Chl was higher in the plants grown in saline medium irrespective of the varietal tolerance to salinity (Seigel and Bjarsh, 1962) and is dependent on salt levels (Romero- Aranda et al, 2001). Higher Chl content did not necessarily translate into higher grain yields (Sharma and Mani, 1997).

The accumulation of osmolytes in plants in response to salinity has been widely reported. Increased accumulation of sugars has been reported in many studies (Dubey and Singh, 1999; Flowers, 2004; Pattangul and Thitisaksakul, 2008). The plants encountering salt stress showed reduction in protein, starch and total carbohydrates and increase in reducing sugars (Joshi, 1984). Saline stress induces proline accumulation which is associated with osmotic adjustment (Stewart and Lee, 1974; Bal, 1975; Larher et al, 1993) in response to the decrease in leaf water potential (Chu et al, 1976). Salinity index of leaf proline showed strong positive relationship with salinity index of yield and is thus a promising index for deploying in breeding programmes for evolving salt tolerant rices (Pandey and Srivastava, 1989; Summart et al, 2010).

However, most studies on screening of crops for salinity tolerance were done under controlled in vitro conditions using single salts, mostly NaCl. In nature the soil solution is a complex mixture of salts; studies involving neutral salts mixtures like NaCl, Na_2SO_4 and $CaCl_2$ have been fewer. Also, the evaluation of tolerance to salinity and alkalinity has been conducted separately by different workers using different sets of genotypes for the two stresses. If conducted at the same time, these were done with limited number of genotypes usually one or few representatives of each. This makes broad generalizations of the comparative effects of salinity and alkalinity tolerance difficult and uncertain. There have been no studies involving the simultaneous screening of a large number of rice genotypes of varying spectrum of salinity as well as alkalinity tolerance to measure the physiological responses. In the present study, we simultaneously screened 8 tolerant, 8 semi-tolerant and 3 sensitive rice genotypes for salinity as well as alkalinity tolerance in saline and highly alkaline soils in lysimeters and analysed the rice plants for chlorophyll and accumulation of selected osmolytes to identify their response to both types of stresses.

MATERIALS AND METHODS

Twenty five rice genotypes representing a range of tolerance to salt response were selected for the study at the Central Soil Salinity Research Institute (CSSRI) experimental station, Karnal, Haryana in northern India. The area is representative of semi-arid sub-tropical India characterized by hot and dry summers and cold winters. Rice was grown in lysimeters (6 m long x 3 m wide x 1.5 m deep) filled with sandy loam soil. One set was salinised by addition of 8.3 g NaCl, 1.5 kg Na_2SO_4 and 2.2 kg $CaCl_2$ $2H_2O$ and another set was alkalinized by addition of sodium bicarbonate (40 kg /lysimeter). The soils were repeatedly wetted and dried for two seasons to ensure uniform equilibrium of salts. Two levels of salinity (average root zone salinity during the entire period of rice growth of EC_e 4 and 8 mS cm^{-1}) and alkalinity (alkalinity- pH 9.5 and 9.8) were achieved. Normal soil (pH 7.3, EC_e 1.2 mS cm^{-1}) was used for control comparisons. The soils were analysed for pH, EC_e, CEC, organic carbon, total N, available P and K as per methods described in Hesse (1971). The salient physico-chemical and fertility properties are listed in table 1.

The rice genotypes ranged from traditional, tall land races to bred dwarfs (Surekha Rao et al, 2008) and are cultivated in different agro-ecological regions in the Indian sub-continent. Of the 25 rice genotypes, except six which gave mixed response, rest of the 19 could be distinctly classified tolerant (T), semi-tolerant (ST), and sensitive (S) groups depending on their absolute yield and relative yield reduction under salinity and alkalinity stress- tolerant (<25% grain yield reduction from normal soil), semi-tolerant (30-50% reduction) and sensitive (>50% reduction) (Surekha Rao et al, 2008). The origin and parentage of the genotypes, and other plant characteristics are given in table 2. The tolerant genotypes used were: CSR1, CSR10, CSR11, CSR21, CSR22, IR36, Jaya, BR4-10; the semi-tolerant genotypes were: CSR13, CSR18, CSR27, CSR29, CSR30, Pokkali, Panvel-1, Co43, and the sensitive genotypes were: P.Bas-1, MI-48, Bas370 and were all obtained from the CSSRI rice germplasm bank.

Rice genotypes were transplanted in three replications in randomized block design, N was applied @ 120 Kg N ha^{-1} as urea in 3 equal splits whereas 40 Kg P_2O_5 ha^{-1} (single superphosphate) and 20 Kg ha^{-1} Zn SO_4 as basal dose. The Chl, proline, sugar and starch contents were analysed in the upper most fully expanded leaf at maximum tillering stage (6 weeks after transplanting) in triplicates. Chlorophyll a and b were analysed in freshly cut leaves by ethanol extraction (Arnon, 1959) by spectrophotometry and expressed on mg leaf fresh wt basis. Starch and sugar were determined by anthrone reagent method (Yoshida et al, 1971) and expressed on dry weight basis. Proline was determined in sulphosalicylic acid extracts (Bates et al, 1973) using ninhydrin and expressed on fresh weight basis. The data on physiological responses of the 19 genotypes was subjected to analysis of variance (ANOVA) using SPSS package; the physiological responses showed highly significant F-values ($p<0.0001$) for the genotypic differences (G), stress environments (E), and G x E interactions (table 3).

RESULTS

The rice genotypes belonged to traditional land races (tall) as well as those bred (medium and dwarf) for high yield and tolerance to salinity and alkalinity (supplementary material, table 1) were found to have a range of tolerance to salinity and alkalinity. Chlorophyll a reduced drastically in all the three tolerance groups at EC_e 4 mS cm^{-1} (by an average of 83.7%) and by 74.1% at EC_e 8 mS cm^{-1}, but there was no reduction at pH 9.5 in any class (Fig. 1). At pH 9.8 however there was a reduction (21.2%) only in the sensitive group. There was reduction in Chl b at EC_e 4 by 52.8% averaged over all the genotypes. At EC_e 8 there was a reduction in Chl b by 33.5 % only in the sensitive group.

Chlorophyll b increased appreciably at pH 9.5 by 75.6, 127.7 and 206.2% in T, ST and S groups. At pH 9.8 it increased appreciably by 179.2 and 186.3% in T and ST; in S group it increased by only 33.5% (Fig. 1). The total Chl content of all the genotypic classes showed a significant decrease of 76.3% under salinity stress at EC_e 4 and 62.3 % at EC_e 8. Under alkalinity stress of pH 9.5 there was an increase in total Chl by 18 % in T, 49.2% in ST and 49.6 % in S genotypes. At pH 9.8 there was significant increase of 61.4 % in T, 54.9% in ST and a slight reduction of 8.8 % in S genotypes (Fig.1) over normal soil. Chlorophyll a/b ratio pattern in T and ST averaged over the genotypes decreased by 65.4% from 3.04 in normal to 1.1 at EC_e 4 and EC_e 8. In sensitive genotypes, it decreased by 67.5 % from 3.47 to 1.13 at EC_e 4 and 8. Under alkalinity stress of pH 9.5 Chl a/b ratio decreased in T and ST by 43.3% to 1.72. In S genotypes it decreased by 65.4% to 1.2. At pH 9.8 it decreased by 53.7 in T and ST to 1.35; in S genotypes it decreased by 38.9% to 2.12.

The leaf sugar content significantly decreased at higher salinity (EC_e 8 mS cm $^{-1}$) in T and ST genotypes by 39.0 and 31.2% but was unaffected in S genotypes. Under alkalinity stress the T and ST genotypes were unaffected but there was an increase in S genotypes at pH 9.5 by 63.5 % and pH 9.8 by 51.0 %. (Fig. 1). The leaf starch content was unaffected by salinity of EC_e 4 in ST and S genotypes and was significantly decreased only in T genotypes even at lower salinity of EC_e 4 by 32.5 % and by 40.5 % at EC_e 8 mS cm $^{-1}$. Alkalinity showed no significant effect on ST and S genotypes; there was no effect on T genotypes at pH 9.5 although at pH 9.8 there was a marginal reduction (22.5%) in T genotypes. Proline content in the leaves consistently and sharply increased with increase in salinity and alkalinity over normal in all the tolerance groups. Averaged over the given classes it increased by 70.2, 109.5, 76.3 and 121.3% at EC_e 4, 8, pH 9.5 and 9.8 respectively.

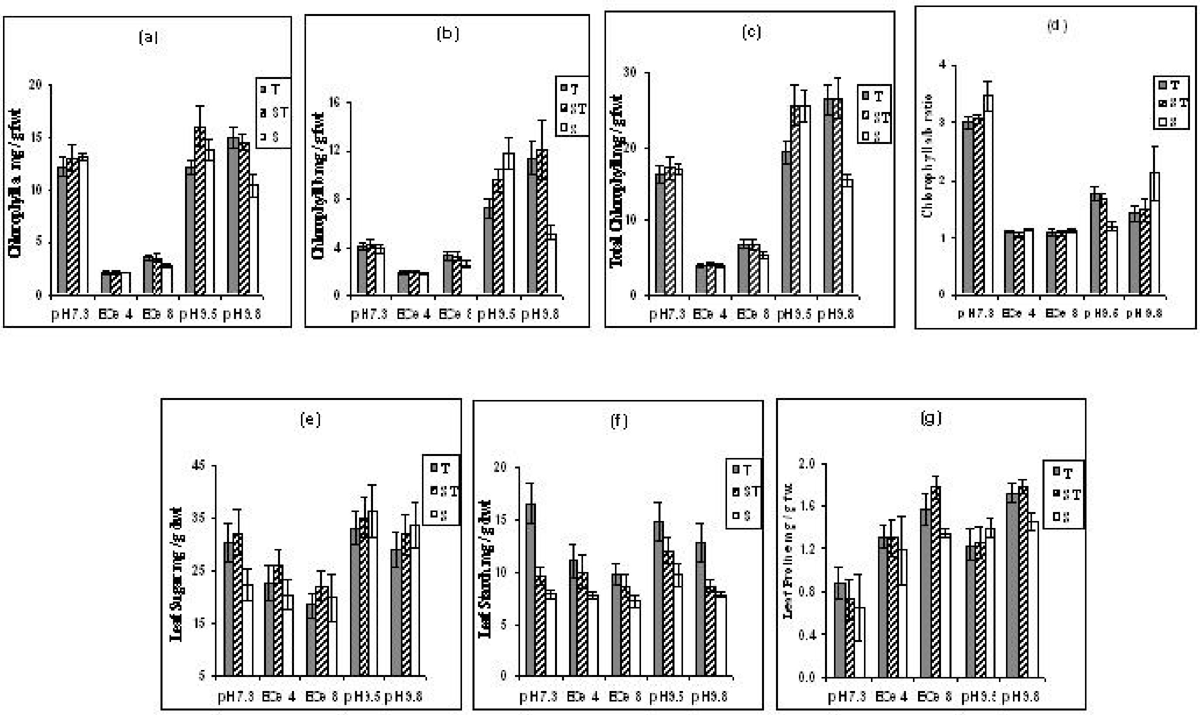

Fig.1: Effect of soil salinity (E Ce) and alkalinity (pH$_2$) levels on an average Leaf Chl a (a), Chl b (b), Total chl (c), Chl a/b ratio (d), Leaf Sugar (e), Leaf Starch (f) and Leaf Proline (g) of T, ST and S rice genotypes.

Table 1. Salient physico-chemical and fertility properties of the experimental soils.

Property	Normal	Saline-1	Saline–2	Alkali -1	Alkali-2
pH (1:2, w/v)	7.3	8.2	8.7	9.5	9.8
ECe (mS cm^{-1})	1.5	4.2 ± 0.7	8.2 ± 1.7	1.1 ± 0.1	1.3 ± 0.2
CEC (cmol 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
Avail. P (kg ha^{-1})	12.0	9.0	17.0	12.0	19.4
Avail. K (kg ha^{-1})	241	179	202	200	225

Table 2. 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 1 (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 3. Chlorophyll and osmolytes comparisons (paired t-test, p=0.05) within a particular salinity or alkalinity level, among different tolerance groups of rice genotypes.

Attribute	Interaction	Df	Sum of squares	Mean square	F value	Pr > F
Total Chlorophyll ($mg\ g^{-1}$ fwt)	G	24	3050.2	127.1	7.2	<.0001
	E	4	23117.1	5779.3	326.8	<.0001
	G x E	96	9859.7	132.7	5.1	<.0001
Leaf Sugar ($mg\ g^{-1}$ dwt)	G	24	18003.7	75.2	32.3	<.0001
	E	4	8456.7	2114.2	90.9	<.0001
	G x E	96	13581.5	141.5	6.1	<.0001
Leaf Starch ($mg\ g^{-1}$ dwt)	G	24	3290.4	137.4	38.3	<.0001
	E	4	1057.5	264.4	75.8	<.0001
	G x E	96	2298.0	23.9	6.7	<.0001
Leaf Proline ($mg\ g^{-1}$ fwt)	G	24	9.7	0.4	19.9	<.0001
	E	4	41.4	10.3	508.2	<.0001
	G x E	96	46.5	0.5	23.3	<.0001

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; with 50% yield reduction at EC_e of 6 mS cm^{-1} (Maas and Hoffman, 1977) and tolerant to alkalinity; some traditional salt tolerant varieties can withstand high pH of upto 10.0 under irrigated conditions (Mishra and Bhattacharya, 1980). Hence the higher alkalinity level of pH 9.8 and salinity level of 8.0 mS cm^{-1} used in the present experiment were realistic enough to differentiate the physiological responses of the tolerant, semi-tolerant and sensitive genotypes of rice.

Chlorophyll content becomes a first indication of responses in different plants subjected to salinity stress (Roy Choudhury and Basu, 2008).Experimental results indicated degradation of Chl a and b due to salinity stress of ECe 4 and 8 mS cm^{-1} in all the tolerance groups which are in agreement with Cha-um et al, (2009) the degradation of Chl a in both the salt tolerant and salt sensitive cultivars and in accordance with Amirjani (2011), who showed that the reduction of chlorophyll a and b was detected after NaCl treatment in leaves. In general, Chl a was not much affected by alkalinity stress while Chl b increased with alkalinity. Both at EC_e 8 and pH 9.8 Chl a and b were more in tolerant varieties than in sensitive ones, although the differences were smaller under salinity and striking under alkalinity stress. This is only in partial agreement with Pandey and Srivastava, (1987) who showed that a soil salinity of 10 mS cm^{-1} EC_e decreased the Chl content and photosynthetic rate in 10 rice cultivars with decrease being smaller in salt resistant cultivars than sensitive ones. In our case, reduction in total Chl was 58.1 % in tolerant and 68.4% in sensitive which is in accordance with the findings of Ghosh et al, (2010) who showed that Nona Bokra (a relatively salt resistant variety), however recorded less loss of chlorophyll than Pokkali (a relatively sensitive variety). A decrease in total Chl was also observed by Krishnamurty et al, (1987) upon irrigation of rice with saline water due to the Chl degradation. The results are in contrast with reports on higher Chl a and b in response to salinity in both tolerant and sensitive genotypes (Misra et al, 1997; Peiris et al, 1993). The decrease in Chl content under stress is a commonly reported phenomenon in other plants and may be due to the membrane deterioration (Mane et al, 2010; Tantawy et al, 2009). Usually there is dominance of Chl 'a' over Chl 'b' in plants but their values become closer with increasing salinity (Mane et al, 2010). Our results on reduction of Chl a/b ratio in salinity as well as alkalinity stress supports the above view. The ratio of Chl a/b was similar in T, ST and S genotypes under salinity stress thus not in agreement with Zhang et al, (2012) who showed higher ratio in tolerant than a sensitive variety.

Limited supply of essential metabolites, e.g., of carbohydrate could retard growth under sub-lethal salinity stress. There is evidence that starch and sucrose pathways are a factor in tolerance to metabolic stresses (Rathert, 1984) and accumulation of sugars is an effective mechanism of osmotic adjustment in non-halopytes (Munns et al, 1982). Sugar content decreased with salinity stress in T and ST genotypes but was not affected in S genotypes; it was stable in T and ST at pH 9.5 and 9.8 but increased in the sensitive group. The accumulation of sugars was higher in tolerant genotypes as compared to the sensitive ones under normal conditions but at salinity stress of EC_e 4-8 or alkalinity stress of pH 9.5-9.8 the differences were not significant. This is in agreement with Aleshin et al, (1984) who showed reduction in sugar content in rice stems and roots with the reduction being more with higher levels of saline stress. Murthy and Raja Rao, (1967); Amirjani, (2011) and Zhang et al, (2012) showed significant increase in sugar content in rice varieties under salt stress. However in our case the sugar content was similar in tolerant and sensitive under stress. The extent of osmotic adjustment via sucrose accumulation probably depends on salt tolerance of the crop. In contrast to moderately sensitive rice, moderately tolerant soybean and tolerant cotton have other more important tolerant mechanisms e.g., proline accumulation (Weimburg et al, 1982) to effect osmotic adjustment at a given salinity. This may explain why the tolerant and semi-tolerant rices in our study did not accumulate sugars while only the sensitive did.

Like sucrose, salinity induced change in total leaf starch has been found to be inversely correlated with salt tolerance of species, intra-specific differences in accumulation have been reported in crops including in rice (Rathert, 1984). The pattern of leaf starch accumulation was consistent and was highest in tolerant, intermediate in semi-tolerant and lowest in sensitive rice genotypes in normal soil as well as under salinity and alkalinity stress. There was a general decrease in starch with salinity and alkalinity stress only in T group but not in ST or S group. Aleshin et al, (1984) also showed that accumulation of starch decreased with increase in salinity. Formation of leaf starch as temporary energy storage available for growth and respiration may be linked with disturbance by NaCl of sucrose metabolism (Rathert, 1984). The function of increased foliar starch for metabolic adaptation to salinity stress is speculative and the early stage is characterized more by inhibited utilization of carbohydrates than by limited carbohydrate supply (Munns et al, 1982).

Proline consistently increased under salinity as well as alkalinity significantly in all the tolerance groups which is in accordance with Summart et al, (2010) who showed that salt stress caused an increase in the accumulation of proline, hence proline was thus a robust indicator of plant stress even at low salinity of EC_e 4 mS cm^{-1} or pH 9.5.

Among the groups, there was higher accumulation of proline in tolerant genotypes under higher salinity (EC_e 8) and alkalinity (pH 9.8) in absolute terms thus supporting the view of Krishnamurty et al, (1987) who found that salt tolerant rice cultivars subjected to NaCl (EC_e 10 dSm^{-1}) stress maintained higher levels of proline than salt-sensitive cultivars. But in relative terms of accumulation over normal soil, proline accumulated 1.8x in tolerant; 2.4x in semi-tolerant and 2.1x in sensitive genotypes at EC_e 8.0. At high pH 9.8, it increased by 2.0x in tolerant; 2.4x in semi-tolerant and 2.2x in sensitive genotypes over normal soil. The salt tolerant rice genotypes accumulated an average ~ 1.5x proline in shoot under salinity while sensitive accumulated ~1.2x (Pandey and Srivastava, 1989). Bal, (1975) reported very high proline and alanine content of wild rice at salinity of 25 mS cm^{-1} than cultivated rice. Proline accumulation is caused by both the activation of its biosynthesis and inactivation of its degradation (Mattioni et al, 1997) and along with sugars, polyols, amino acids and quaternary ammonium compounds have been most associated with osmotic adjustment in higher plants in response to osmotic stress (Chu et al, 1976; Cha-um et al, 2009; Flowers, 2004, Mattioni et al, 1997).

In conclusion, the salt tolerant genotypes of rice maintained higher levels of chlorophyll a and b, starch and proline under higher salinity and alkalinity stress which could have contributed to their salt tolerance (Zhang et al, 2012) and indicates that they are useful as robust screening criteria for both of higher salinity and alkalinity tolerance.

ACKNOWLEDGEMENTS

The authors are grateful to Director, CSSRI, Karnal for providing the facilities for the investigations; to Dr. P.C. Sharma and Dr. Ali Qadar for helpful advice and useful discussions.

REFRENCES

Aleshin, E.P., Vorobev, N.V. and Zhurba, T.P. (1984) The physiological reasons for the different levels of salt resistance in rice varieties. Doklady Vseroyuznoi Ordena Lenina I Ordena Trudovogo Kransnogo Znameni Akademii, Sel'skokhozyaistvenuykh Nauk Imeni V.I. Lenina, **8,** 3-5.

Amirjani, M.R. (2011) Effect of salinity stress on growth, sugar content , pigments and enzyme activity of rice. *Inernational Journal of Botany*, **7(1)**, 73-81.

Arnon, D. (1959) Copper enzymes in isolated chloroplasts. Polyphenoloxidese in *Beta vulgaris*. *Plant Physiology*, **24**, 1-15.

Ashraf, M. and Harris, P.J.C.(2010) Potential biochemical indicators of salinity tolerance in plants. *Plant Science*, **166(1)**, 3-16.

Bates, L., Waldren, R. P. and Tearo, I. D. (1973) Rapid determination of free proline for water stress studies. *Plant and Soil*, **39**, 205-207.

Bal, A. R. (1975) A note on the comparative study of free amino acids content between wild salt tolerant rice and cultivated rice varieties. *Current Science*, **44**, 194-195.

Cha-Um., Supaibulwattana, S. K. and Kirdmanee, C. (2009) Comparative effects of salt stress and extreme pH stress combined on glycinebetaine accumulation, photosynthetic abilities and growth characters of two rice genotypes. *Rice Sci*, **16**, 274-282.

Chu, T. M., Aspinall, D. and Paleg, L. C. (1976) Stress metabolism VII. Salinity and proline Accumulation in barley. *Australian Journal of Plant Physiology*, **3**, 219-228.

Dubey, R. S. and Singh, A. K. (1999). Salinity induces accumulation of soluble sugars and alters the activity of sugar metabolizing enzymes in rice plants. *Biologia Plantarum*, **42**, 233-239.

Flowers, T. J. 2004. Improving crop salt tolerance. *Journal of Experimental Botany*, **55**, 307-319.

Flowers, T. J. and Yeo, A. R. (1981) Variability in the resistance of sodium chloride salinity within rice (*O. sativa* L.) varieties. *New Phytologist*, **88**, 363-373.

Ghosh, N., Adak, M. K., Ghosh, P. D., Gupta, S., Sengupta, D. N.and Mandal, C. (2011) Differential responses of two rice varieties to salt stress. *Plant Biotechnology reports*, **5(1)**, 89-103.

Hesse, P. R. (1971) A Text Book of Soil Chemical Analysis, John Murray, London.

Hossain, M. and Fischer, K. S. (1995) Rice research for food security and sustainable agricultural development in Asia. Achievements and future challenges. *Geojournal*, **35,** 286-298.

Joshi, Y. C. (1984) Effect of salinity stress on organic and mineral constituents in the leaves of pigeon pea (*Cajanus Cajan* L. Cv. C-II). *Plant and Soil*, **82,** 69-76.

Khan, M. A. and Abdullah, Z. (2003) Salinity-sodicity induced changes in reproductive physiology of rice (*Oryza sativa*) under dense soil conditions. *Environmental and Experimental Botany*, **49**, 145-157.

Krishnamurty, R., Anbazghan, M. and Bhagwat, K. A. (1987) Effect of sodium chloride toxicity on chlorophyll breakdown in rice. *Indian Journal of Agricultural Sciences*, **57**, 567-570.

Kuchanur, P.H., Patil. S.G., Dronavallin., Pattar, P.S. and Reddy, B.G.M. (2006) Screening of rice (*Oryza sativa*) genotypes for salt tolerance in saline Vertislos of Tungabhadra Command. *Indian Journal of Agricultural Sciences*, **76,** 286-288

Larher, F., Leport, L., Petrivalsky, M.and Chappart, M. (1993) Effectors for the osmoinduced proline response in higher plants. *Plant Physiology and Biochemistry,* **31,** 911-922.

Mass, E. V. and Hoffman, G. J. (1977) Crop salt tolerance-current assessment. *Journal of Irrigation and Drainage Division, Proceedings of American Society of Civil Engineers,* **103**, 115-134.

Mane, A.V., Karadge, B.A. and Samant, J.S. (2010) Salinity induced changes in photosynthetic pigments and polyphenols of Cymbopogon Nardus (L.) Rendle. *Journal of Chemical and Pharmaceutical Research,* **2**, 338 -347.

Mattioni, C., Lacerenza, N. G., Troccoli, A., De Leonardis, A. M. and Di Fonzo, N. (1997) Water and salt stress-induced alterations in proline metabolism of *Triticum durum* seedlings. *Physiologia Plantarum*, **101,** 787–792.

Mishra, B. and Bhattacharya, R. K.(1980) Varietal tolerance to alkalinity in rice. Proc. Intnl. Symp. Salt affected soils. Karnal, India: pp. 502-507.

Misra, A. N., Sahu, S. M., Misra, M., Singh, P., Meera, I., Das, N., Kar, M. and Sahu, P. (1997) Sodium chloride induced changes in leaf growth, and pigment contents in two rice cultivars. *Biologia Plantarum*, **39,** 257-262.

Munns R, Geenway H, Delane R, Gibbs J. 1982. Ion concentration and carbohydrate status of the elongating leaf tissue of *Hordeum vulgare* growing at high external NaCl. II. Cause of growth reduction. *J Exp Bot* , **33**: 574-583.

Murthy, K. S. and Raja Rao Ch.N**.** (1967) Studies on salt tolerance in rice. *Oryza,* **4,** 42-47.

van Nguyen, N. and Ferrero, A. (2006) Meeting the challenges of global rice production. *Paddy and Water Environment*, **4**, 1-9.

Pandey, U. K. and Srivastava, R. D. L. (1987) Physiological studies on salt tolerance of rice genotypes – yield and yield attributes. *Indian Journal of Plant Physiology*, **30,** 308-310.

Pandey, U. K. and Srivastava, R. D. L. (1989) Salinity index in relation to nitrate reducatase activity and proline accumulation in paddy genotypes. *Indian Journal of Plant Physiology*, **32**, 175-177.

Pattanagul, W. and Thitisakasakul, M. (2008) Effect of salinity stress on growth and carbohydrate metabolism in the rice cultivars (*Oryza sativa.*L.) cultivars differing in salinity tolerance. *Indian Journal of Experimental Biology*, **46,** 736-742.

Peiris, B.D. and Ranasinghe, A. (1993) Effect of sodium chloride salinity on chlorophyll content in rice (*O. sativa* L.) leaves. *Indian Journal of Plant Physiology*, **35**, 257-258.

Qadar, A. (1988) Differential sodicity tolerance of growth and yield parameters in genotypes of rice (*O. sativa*.L.). *Indian Journal of Agricultural Sciences*, **51**, 607-611.

Rathert, G. (1984) Sucrose and starch content of plant parts as a possible indicator for salt tolerance. *Australian Journal of Plant Physiology*, **11**, 491-495.

Romero-Aranda, R., Soria, T. and Cuartero, J. (2001) Tomato plant-water uptake and plant-water relationships under saline growth conditions. *Plant Science*, **160**, 265-272.

Roy Choudhury, A. and Basu, S. (2008) Over expression of an abiotic stress responsive plant protein in bacteria E.coli. *African Journal of Plant Biotechnology*, **7,** 3231-3234.

Seigel, O. and Bjarsh, H. J. (1962) Uberdie Wirkung Von Chloridunal sulphationen auf den stoff weechsel von tomaten seleria and Reben-Garten Bawissenchaf, **27**, 5-16.

Sharma, J. P. and Mani, S. C. (1997) Analysis of biochemical parameters at boot stage in rice. *Indian Journal of Genetics and Plant Breeding* **57**, 238-242.

Stewart, G. R. and Lee, J. A. (1974) The role of proline accumulation in halophytes. *Planta*, **12**, 279-289.

Sudhir, P. and Murthy, S.D.S. (2004) Effects of salt stress on basic processes of photosynthesis. *Photosynthetica*, **42**, 481-486.

Summart, J., Thanonkeo, P., Panichajakul, S., Prathepha, P. and McManus, M.T. (2010) Effect of salt stress on growth, inorganic ion and proline accumulation in Thai aromatic rice, Khao Dawk Mali 105, callus culture. *African Journal of Biotechnology*, **9,** 145-152.

Surekha Rao, P., Mishra, B., Gupta, S. R. and Rathore, A. (2008) Reproductive stage tolerance to salinity and alkalinity stresses in rice genotypes. *Plant Breeding*, **127**, 256-261.

Tantawy, A. S., AbdelMawgoud, A. M. R., ElNemr, M. A. and Chamoun, Y. G. (2009) Alleviation of Salinity Effects on Tomato Plants by Application of Amino Acids and Growth Regulators. *European Journal of Science Research*, **30**, 484 – 494.

Tyagi, N. K. and Minhas, P. S. (1998) Agricultural Salinity Management in India. Central Soil Salinity Research Institute, Karnal, India: pp. 526.

Weimburg, R., Lerner, H.R. and Poljakoff-mayber, A. (1982) A relationship between potassium and proline accumulation in salt stressed *Sorghum* *bicolor*. *Physiologia Plantarum*, **55**, 5-10.

Yoshida, S., Forno, D. A. and Coek, J. H. (1971) Laboratory manual for physiological studies of rice, pp 23-24. Internationl Rice Research Institute, Manila, Philippines.

Zhang, Zhen-hua., LIU, Qiang., Song, Hai-xing., Rong, Xiang-min. and Abdelbagi, M. Ismail. (2012) Responses of different rice (*Oryza sativa* L.) genotypes to salt stress and relation to carbohydrate metabolism and chlorophyll content. *African Journal of Agricultural Research*, **7(1)**, 19-27.