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ORIGINAL ARTICLE

Differential Response of Two Scented Indica Rice (Oryza sativa) Cultivars under Salt Stress

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Present report deals with the effect of varying (0 – 200 mM NaCl) salt stress on two popular scented non-basmati type indica rice cultivars, namely Indrayani and Ambemohar on germination and growth and biochemical parameters. In the present investigation the effect of increasing salt stress was seen on germination, biomass production and biochemical parameters including total protein content, proline accumulation, starch content, polyphenols levels, and reducing and non-reducing sugars. Contrasting behavior was evidenced in both the cultivars in terms of germination rate and biomass production at seedling and early vegetative growth level. Salt stress-induced proline accumulation was observed in both the cultivars, however, with much higher extent of proline accumulation in Ambemohar than Indrayani. A salinity stress of 200 mM NaCl resulted into 305% higher proline content than the control plants of Ambemohar against 222% higher proline in Indrayani at the same stress level. Similarly protein content was also higher in Ambemohar than Indrayani at the highest stress level used in this study. Contrasting results were seen in terms of starch content amongst both the cultivars, where continuous decrease with increasing salt stress was observed in Indrayani, on the other hand, an increase in starch content was evident in Ambemohar under the influence of NaCl-induced salt stress. These finding clearly indicates the comparably higher salt tolerant nature of Ambemohar than Indrayani which might be attributed to higher proline, protein and starch content than Indrayani cultivar under salt stress.

Key words: *Scented rice/ salt stress/ proline/ protein/ polyphenols*

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Various abiotic stresses including high or low temperature, water scarcity, high salinity and heavy metals exert drastic antagonistic effects on crop metabolism and thereby plant growth, development and ultimately crop productivity. Amongst these, soil salinity is a major factor limiting the crop production globally (Kumar *et al.*, 2010). Soil salinity affects large areas of the world cultivated land causing significant reductions in crop yield (Tavakkoli *et al.*, 2011). In Asia alone, 21.5 million

ha of land area is thought to be salt-affected, with India having 8.6 million ha of such area which constitutes a major part of problem soils in India (Sahi *et al.*, 2006). However, improvement in salt tolerance of crop plants remains elusive, due to the fact that salinity affects almost every aspect of the physiology and biochemistry of plants (Cuartero *et al.* 2006) at both whole plant and cellular levels (Murphy and Durako 2003). Generally, soil salinity affects plants through osmotic effects, ion-specific effects, and oxidative stress (Pitman and Lauchli, 2002).

Rice is one of the world's most important cereal crops with exceptional agricultural and economic importance as being a staple food for more than 50% population worldwide and Asian farmers produce more than 90% of the total rice, with two countries India and China, growing more than half of the total crop (IRRI, 2011). In addition to its food values and economic importance rice, with its relatively small genome size together with its complete genome sequence (Sasaki *et al.*, 2005), is considered as a model monocot system for various biotechnological, metabolic, genetic engineering and functional genomics development studies worldwide (Bajaj and Mohanty, 2005).

However, the yield of rice, especially Asian rice (sativa), is highly susceptible to salinity (Munns and Tester 2008). In India and especially in coastal rice fields of Maharashtra state, soil salinity is a major stress that reduces the rice productivity to a great extent (Kumar *et al.*, 2008).

Salinity is detrimental to the various processes of crops such as seed germination, seedling growth and vigor, vegetative growth, flowering and fruit set and ultimately it causes diminished economic yield and also quality of produce (Sairam and Tyagi 2004). In response, plants have developed a number of mechanisms to counteract high salt stress such as mineral ions homeostasis and accumulation of compatible solutes such as proline (Sairam *et al.*, 2005). Moreover, salt stress responses of plants may depend upon salt type, concentration, and genotype. Therefore, screening for salt-stress tolerant genotypes in important crops such as rice will help in ensuring future crop production. In addition, studying differential responses of genotypes with contrasting stress tolerances will help reveal the underlying salt stress tolerance mechanisms (Kumar *et al.*, 2008).

The present investigation was aimed to study the comparative effects of NaCl stress towards germination, plant growth and various biochemical parameters including total proteins, sugars and carbohydrates, starch and proline accumulation in two local highly popular indica scented non-basmati type rice genotypes, namely Ambemohar and Indrayani.

MATERIALS AND METHODS

Plant material and salt treatments

Two local (Maharashtra, India) scented rice (*Oryza sativa* L.) cultivars namely Indrayani and Ambemohar were selected for this study. Certified seeds were obtained from the Regional Rice Research Station, Karjat (Maharashtra, India). Seeds of both the cultivars were surface sterilized with 0.1% mercuric chloride for 10 min and then washed several times with sterile distilled water. Twenty five seeds of each cultivar (cv.) were sown in the Petri dishes (10 cm diameter, Axygen, India) containing germination paper. The experiment was conducted in laboratory at the room temperature with 12 h daylight. Every day 5 ml of distilled water (with or without varying NaCl levels, i.e. 50, 100, 150, 200 and 300 mM) was applied per Petri dish and all the observations were recorded on the 21st day after

germination. The physiological and biochemical studies were carried under the different salinity levels and all the experiments were carried out in triplicate.

Germination studies and plant growth analysis

Total germination was expressed as a percentage of the control for each variety. The germination percentage, root length, shoot length, and root/shoot ratio of seedling were recorded on the 21st day of germination.

Biochemical analyses

Determination of proline content

Free proline content was estimated by following the method of Bates *et al.* (1973). Fresh 0.5 gm root and shoot samples were homogenized in 5 ml of 3% sulphosalicylic acid using a mortar and pestle. About 2 ml of extract was taken in test tube and to it 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent were added. The reaction mixture was boiled in a water bath at 100°C for 30 min. After cooling the reaction mixture, 4 ml of toluene was added. After thorough mixing, the chromophore containing toluene was separated and absorbance of red color developed was read at 520 nm against toluene black on UV-visible spectrophotometer (Chemito, UV-2600). The proline concentration was determined using calibration curve and expressed as mg proline per g fresh weight of tissue.

Determination of total protein content

Proteins were estimated using Lowry *et al.* (1951) method. Fresh samples (250 mg) were homogenized in 2.5 ml of phosphate buffer (pH 7.0). The extract was centrifuged at 5000 g for 15 min at 4°C and the supernatant was transferred to a tube containing a mixture of 20 ml acetone and 14 ml β-Mercaptoethanol for precipitation of protein. The sample tubes were stored at 0°C for 5 h and then centrifuged at 10000 *g* for 20 min. The supernatant was discarded and the pellet was dissolved in 2.5 ml 1 N sodium hydroxide solution. Aliquot of 0.2 ml from this sample was used to prepare the reaction mixture. The intensity of blue color developed was recorded at 660 nm and protein concentration was measured using bovine serum albumin as standard.

Determination of Total Phenols

Total phenol contents were estimated by following Malick and Singh (1980). Total phenols were extracted from 500 mg of fresh roots and shoot tissues separately in 80% (v/v) ethanol and estimated by Folin-Ciocalteau reagent. The absorbance of the reaction was measured at 650 nm wavelength on UV-visible spectrophotometer (Chemito, UV-2600). Total phenols were calculated by using standard graph of catechol.

Determination of reducing and non-reducing sugar levels

Reducing sugars were estimated using Dinitrosalicylic acid (DNSA) reagent by following the method of Miller (1972). Fresh shoots (0.1 g) of plants with or without salt concentrations (0-200 mM NaCl) were homogenized in hot 80% ethanol. The extract was centrifuged at 5000 *g* for 15 min at room temperature and the supernatant was evaporated by keeping in water bath at 80°C and sugars were dissolved by adding 10 ml distilled water. Reducing sugars were estimated by using DNSA reagent colorimetrically at 530 nm wavelength and calculated from graph plotted using glucose as a standard.

Non-reducing sugars were estimated using anthrone reagent (Hedge and Hofreiter, 1962). Fresh shoots (0.25 g) were hydrolysed separately by keeping in boiling water bath for 3 h with 2.5 N HCl (5 ml) and was neutralized with Na_2CO_3 after

cooling it to room temperature. Volume was made up to 100 ml and centrifuged at 5000 *g* for 15 min at room temperature. Non-reducing sugars were estimated spectrophotometrically at 630 nm wavelength on UV-visible spectrophotometer (Chemito, UV-2600) and calculated from graph plotted using glucose as a standard.

Determination of Starch

Starch was estimated using anthrone reagent by following the method given by Thayumanavan and Sadasivam (1984). Fresh roots and shoots (250 mg each) were separately homogenized in hot 80% ethanol (v/v) to remove sugars. Residue was retained after centrifugation at 5000 x *g* for 15 min at room temperature. The starch was extracted by 52% perchloric acid at 0ºC for 20 min. Starch was estimated by using anthrone reagent spectrophotometrically at 630 nm wavelength on UV-visible spectrophotometer (Chemito, UV-2600) and calculated from graph plotted using glucose as a standard.

Statistical analyses

Each Petri dish was considered as replicate and all the treatments were repeated three times and data are expressed as mean ± standard error (S.E.). All the statistical analyses were done using MSTAT-C statistical software package.

RESULTS AND DISCUSSION

Effect of NaCl stress on germination and early vegetative growth:

The most critical stage in seedling establishment is usually considered as seed germination which consequently determines the successful crop production (Almansouri *et al.* 2001; Kumar *et al.*, 2007). Understanding the responses of plants at these stages is particularly important for elucidating the mechanisms of salt resistance or sensitivity in plants and their survival. In the present report, NaCl-induced salt stress significantly influenced the germination in both the rice cultivars and germination percentage was reduced gradually with increasing salt stress from 0 – 300 mM NaCl (Table 1). The results clearly showed that the percentage emergence of both varieties was reduced by increasing salt levels. However, noticeable difference was evidenced amongst both the cultivars in terms of germination rate. Up to 50 mM NaCl, 100% germination was observed in both varieties; however beyond that level considerable difference was seen with more pronounced reduction in Indrayani than the Ambemohar. Increasing salt stress resulted in gradual decrease in shoot and root length with more adverse effects on shoots growth (Table 1). Amongst these two scented rice cultivars Ambemohar showed better shoot growth and therefore lower root/shoot ratio as compared to Indrayani at high salinity stress levels. The germination and biomass production at early seedling growth level clearly showed that Ambemohar was affected with much lesser extent than Indrayani by NaCl stress. After germination, the plants beyond 200 mM NaCl stress could not grow further and after a few days turned brown therefore, biochemical analyses were done only up to 200 mM NaCl stress. Results of the previous studies by Sumithra *et al.* (2006) and Kumar *et al.* (2009) demonstrated that the salt tolerant cultivars produce greater biomass than salt sensitive mungbean and rice cultivars respectively, when irrigated with NaCl dominated waters.

Our results are in agreement of these findings and it was concluded that the severity of salinity antagonism to the germination and normal growth of plant as indicated by germination percentage, shoot length, root

length, and root/shoot ratio of seedlings was higher in the rice cultivar Indrayani indicating that this cultivar is comparably salt sensitive than Ambemohar.

Table 1. Effect of different concentrations of NaCl on germination and growth parameters at seedling level in rice cultivars

Rice Cultivar	NaCl stress (mM)	Germination percentage Mean ± S.E.	Root length (cm) Mean ± S.E.	Shoot length (cm) Mean ± S.E.	Root/shoot ratio
Indrayani	0 (Control)	100 ± 1.0	14.5 ± 1.2	10.5 ± 2.1	1.38
	50	100 ± 2.2	12.0 ± 1.3	8.1 ± 1.3	1.48
	100	96 ± 1.8	11.5 ± 0.9	7.2 ± 1.0	1.60
	150	88 ± 2.2	6.8 ± 0.6	3.8 ± 0.4	1.79
	200	68 ± 1.7	4.5 ± 0.3	2.2 ± 0.2	2.05
	300	48 ± 1.6	1.8 ± 0.1	0.8 ± 0.1	2.25
Ambemohar	0 (Control)	100 ± 0.8	10.3 ± 1.5	11.0 ± 1.2	0.94
	50	100 ± 1.5	8.2 ± 1.1	7.8 ± 1.1	1.05
	100	100 ± 2.0	8.7 ± 0.8	7.2 ± 0.9	1.21
	150	96 ± 2.5	8.1 ± 0.9	6.1 ± 0.6	1.33
	200	88 ± 1.6	7.5 ± 1.0	3.9 ± 0.4	1.92
	300	68 ± 1.2	4.6 ± 0.3	3.8 ± 0.2	1.21

Table 2. Effect of different concentrations of NaCl on proline content at seedling level in rice cultivars

	Total proline content in rice cultivars (mg/g fresh weight) Mean ± S.E.	
NaCl stress (mM)	Indrayani	Ambemohar
0 (Control)	70.15 ± 2.3 (100)	77.89 ± 3.9 (100)
50	77.89 ± 1.8 (111)	128.51 ± 6.8 (165)
100	109.87 ± 3.9 (156)	140.20 ± 10.2 (180)
150	124.61 ± 5.7 (178)	214.25 ± 12.7 (275)
200	155.82 ± 8.3 (222)	237.62 ± 13.9 (305)

The values in parentheses shows the increase in proline content by considering proline content in control plants as 100%.

Effect of NaCl stress on proline content:

Rapid accumulation of free proline is a typical response to salt stress (Parida *et al.* 2008). Similar responses were observed in the present investigation and salinity stress resulted into a sharp increase in proline content irrespective of the cultivars. However, the rate of salt stress-induced proline accumulation was considerably higher in Ambemohar than its counterpart, Indrayani. In control plants the proline content was almost similar in both the varieties, however, as the magnitude of salinity stress increased, the rate of proline accumulation was observed much higher in Ambomohar (305 % of control) as compared to Indrayani (222% of control) and at 200 mM NaCl stress (Table 2).When exposed to high salt content in soil, many plants have been observed to accumulate high amounts of proline, in some cases several times the sum of all other amino acids (Kumar *et al.*, 2009). Proline is a known osmo-protectant, and plays an important role in osmotic balancing, protection of sub-cellular structures, enzymes and in increasing cellular osmolarity (turgor pressure) that provide the turgor necessary for cell expansion under stress conditions (Matysik *et al.* 2002; Sairam and Tyagi 2004). The present results also support

these hypotheses and clearly suggested the positive relation of higher proline accumulation with comparably better salt tolerance nature of scented rice cultivar Ambemohar.

Effect of NaCl stress on protein content:

Under control (non-saline) conditions, Indrayani showed higher protein content than Ambemohar (Table 3). However, a continuous decrease in protein content with increase in salt stress was observed in Indrayani cultivar, where around 64% reduction was observed in plants under 200 mM NaCl stress as compared to the control plants. On the other hand, the protein content was increased up to 50 mM in Ambemohar and even at 200 mM NaCl stress level the reduction in protein content was a mere 13% as compared to a whopping 64% in Indrayani (Table 3). These observations showed that Ambemohar could withstand salt stress in a better way than the other cultivar in terms of total protein content. Salinity stress is reported to trigger the expression of several osmo-responsive proteins in rice tissues and a correlation has been found between greater accumulations of these stress proteins in halotolerant, compared to salt-sensitive rice genotypes (Chourey *et al.* 2003; Kumar *et al.* 2009). The proteins that accumulate under salt stress conditions may provide a storage form of nitrogen that is re-utilized in post-stress recovery (Singh *et al.* 1987) and also play a role in osmotic adjustments. Our results are in harmony of these findings and indicated that the higher protein content in Ambemohar than Indrayani under salt stress conditions may be one of the factors contributing towards the better salt stress tolerance of the earlier cultivar.

Table 3. Effect of different concentrations of NaCl stress on total proteins content at seedling level in the local cultivar of rice

	Total protein content (mg/g fresh weight) Mean ± S.E.		**Total Phenol content (mg/g fresh weight) Mean ± S.E.**	
NaCl stress (mM)	**Indrayani**	**Ambemohar**	**Indrayani**	**Ambemohar**
0 (Control)	461.70 ± 3.9	287.48 ± 5.2	11.58 ± 0.7	19.74 ± 1.2
50	349.42 ± 7.8	424.37 ± 8.9	13.16 ± 1.5	16.45 ± 1.8
100	299.50 ± 5.7	349.40 ± 13.5	16.45 ± 2.3	19.16 ± 2.1
150	249.64 ± 6.6	312.26 ± 12.1	19.74 ± 2.5	22.08 ± 0.5
200	212.13 ± 7.2	249.67 ± 7.3	26.32 ± 2.9	23.01 ± 0.4

Table 4. Effect of different concentrations of NaCl stress on reducing and non-reducing sugars, and starch content in rice cultivars

	Reducing sugar content (mg/g fresh weight) Mean ± S.E.		**Non-reducing sugar content (mg/g fresh weight) Mean ± S.E.**		**Starch content (mg/g fresh weight) Mean ± S.E.**	
NaCl stress (mM)	**Indrayani**	**Ambemohar**	**Indrayani**	**Ambemohar**	**Indrayani**	**Ambemohar**
0 (Control)	120.5 ± 4.2	40.17 ± 2.8	124.4 ± 5.9	305.4 ± 12.8	617.2 ± 25.2	283.9 ± 8.7
50	160.7 ± 8.4	50.22 ± 3.2	101.8 ± 3.9	271.4 ± 10.4	345.6 ± 15.6	382.7 ± 9.6
100	200.9 ± 12.5	70.31 ± 3.9	67.86 ± 4.2	124.4 ± 8.7	176.9 ± 12.8	481.4 ± 14.1
150	270.3 ± 10.8	80.35 ± 7.1	33.93 ± 2.8	101.8 ± 5.8	102.9 ± 8.9	580.2 ± 17.5
200	281.2 ± 10.9	90.39 ± 6.2	11.31 ± 0.9	45.24 ± 2.6	49.38 ± 2.4	650.1 ± 16.2

Effect of NaCl stress on total phenol content:

The genetic variations among the various crop plants are useful in providing a valuable tool in the selection of cultivars with desirable traits (Misra and Dwivedi, 2004). Anil *et al.* (2005) emphasized the importance of identifying the traits that impart salt tolerance to rice lines and advocated that it would have significant agronomic consequences. The results presented in Table 3 makes it clear that total phenol content was gradually increased with progressing salt stress in Indrayani, however contrasting results were evidenced in Ambemohar with much lower magnitude of increase in phenol contents in the later. Similar results are reported by Parida *et al.* (2002), and author reported increase in polyphenol levels in *Brugiera parviflora* under salt stress.

Effect of NaCl stress on sugars and starch content:

Reducing sugar content was higher in Indrayani cultivar than Ambemohar with or without salinity stress. On the other hand Ambemohar showed higher non-reducing sugar level than Indrayani, irrespective of the salinity stress level (Table 4). Reducing sugar content was increased in both the cultivars with continuous progression of NaCl stress, without much striking difference amongst them. However considerable amount of differential response was observed in terms of non-reducing sugars, where Ambemohar showed higher level of these sugar contents than Indrayani at varying salt stress.

Most striking results were seen in starch content where a sharp decrease was observed in Indrayani plants with increasing salt stress from 50 – 200 mM NaCl, whereas starch content was increased significantly under the influence of salt stress in Ambemohar plants.

Similar to the results of present investigation, Kafi *et al.* (2003) observed higher starch content in salt tolerant wheat genotype at 300 mM NaCl stress. Dubey and Singh (1999) obtained the similar kind of results and concluded that the starch content was reduced with much higher magnitude in salt sensitive rice cultivars than that of salt tolerant ones up to moderate salinity stress (150 mM). Starch is an important component of plant tissues and accumulates in leaves as a temporary reserve form of carbon and is the principal component of dry mass accumulated in mature leaves, hence the accumulation of more starch in Ambemohar may be seen as the protective mechanism during stress conditions.

From the results obtained in the present investigation, we can conclude that overall Ambemohar showed better tolerance to salt stress than Indrayani, with a lesser extent of antagonistic effect of NaCl on germination and biomass production at seedling stage. In addition Ambemohar showed higher proline, protein and starch content with lesser polyphenol levels under varying salt stress level than Indrayani and all these biochemical parameters might have played an important role in its salt tolerance nature.

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