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Effects Of Trehalose and Proline Upon Sodium Chloride Salinity Stress In Rice Cultivars

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ABSTRACT

The objective of this work is to study the effects of trehalose and proline (osmoprotectants) upon sodium chloride salinity stress in rice cultivars by analyzing total chlorophyll, total soluble sugars and proline contents. The experiment was carried out in two parts. First part of the experiment was to study the effects of salinity at seedling stage with out osmoprotectants treatment and the second part was related to counteract the influence of salinity through the treatments of osmoprotectants. Rice seedlings of fifteen days were treated for 6 h. with 1mM conc. of two osmoprotectants solutions, proline and trehalose. After incubation period treated seedlings were removed and were kept for 72 h in 100, 150 and 200mM NaCl solution. For control no treatment of osmoprotectans as well as not subjected to salinity stress was given. After 72 h seedlings were washed thoroughly with tap water followed by distilled water and were analyzed for total chlorophyll, total soluble sugars and proline .Same procedure was followed for first part of research excluding osmoprotectant treatments. Osmoprotectant treated rice seedlings survived better than non treated seedlings under salt stress and also one more highlight is that even sensitive cultivar (GR-3) also survived in good condition under salt stress than tolerant cultivars (Jaya,Dandi,CSR-27). Among both osmoprotectants trahalose served as potential osmoprotectant than proline.

Key words: *Trehalose, Proline, salt stress, biochemical parameters, rice seedlings*

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INTRODUCTION

Osmotic adjustment in plants subjected to salt stress can occur by the accumulation of high concentrations of either inorganic ions or low molecular weight organic solutes. These solutes of low molecular weight are nontoxic even at high concentrations. Osmoprotectants therefore, play important roles in adaptation against several environmental stresses such as temperature, salt, drought and chilling. Although these play an important role in higher plants grown under saline conditions their relative contribution, varies among species and cultivars. The compatible osmolytes generally found in higher plants are low molecular weight sugars, organic acids, polyols and nitrogen containing compounds, amino acids, proteins and quaternary ammonium compounds (Ashraf and Harris, 2004)

Plants accumulate a number of osmoprotective substances in response to sodium chloride stress. Some of the proteins made during stress synthesize substances believed to serve as osmoprotectants (Delauney and Verma, 1990; Bartels *et al*., 1991). Sodium chloride stressed rice (*Oryza sativa* L.) accumulates polyamines (Krishnamurthy and Bhagwat, 1989) and proline (Alia Saradhi, 1993). When administered externally, these molecules have been found to protect plants from some of the damage that is caused by drought or excess salinity (Kavi kishor, 1989; Genard *et al.,* 1991; Krishnamurthy, 1991). Other plants have been found to accumulate common sugars (Kameli and Lorel, 1993), polyols (Loescher *et al*., 1992; Alexander *et al.,* 1994), or in some cases less common sugars such as trehalose (Fougere *et al.,* 1991; Drennan *et al.,* 1993). It is generally assumed that proline, polyols and sugars serve as physiologically compatible solutes that increase as to maintain a favorable osmotic potential between the cell and its surroundings (Pollard and Wynjones, 1979). There is additional evidence that high concentrations of these substances stabilize some macro molecules or molecular assemblies, thus decreasing the loss of either enzymatic activity or membrane integrity that occurs when water is limiting (Schwab and Gaff, 1990).

Osmoprotective substances accumulate at specified cellular locations but do not through out the plant and are needed to protect different types of molecules or cellular components under stress conditions. Therefore structurally different osmoprotectants choose for their effects on salinity stress in rice (Garcia *et al*., 1997).

Proline and trehalose, both osmolytes are structurally as well as functionally different and effect on a number of sodium chloride sensitive physiological and biochemical processes in rice. Garcia *et al.* (1997) studied the effects of proline and trehalose on different physio and biochemical processes, by giving treatment separately to the sodium chloride stressed rice seedlings and noticed that trehalose protects rice seedlings better than proline. Proline promotes leaf growth only fifteen percent where as trehalose forty five percent. They identified trehalose as a potential osmoprotectant when compared to the effect of proline and their role in salt stress. They found that proline either has no effect or in some cases exasperates the effect of NaCl on growth inhibition, chlorophyll loss and induction of a highly sensitive marker for salt stress, by contrast low to moderate concentrations of trehalose reduced Na^+ accumulation and growth inhibition. Butt higher concentration of (10 mM) prevents NaCl induced loss of chlorophyll in blade, prevents root integrity and enhance growth. Finally they concluded that during osmotic stress trehalose or carbohydrates might be more important for rice than proline.

MATERIALS AND METHODS

Crop : Rice,

Varieties : V1- Jaya (Tolerant), V2 - Dandi (Tolerant), V3 - CSR – 27 (Tolerant), V4 - GR – 3 (Susceptible)

Experimental materials

Experiment was conducted during the year 2005-06 in the Department of Biochemistry under controlled environmental conditions. Four varieties of rice *viz* Jaya, CSR-27, Dandi and GR-3 differing in degree of susceptibility to salt stress were procured from Main Rice Research Station, AAU, Navagam, Gujarat.

Experiment I

Treatments: Seedlings (15 days after germination) of above four varieties were treated with 100mM, 150 mM and 200 mM levels of sodium chloride solution for 24, 48 and 72 h. For control no saline solution was applied

Sample collection: Samples were collected at 24, 48 and 72 h for analysis of total chlorophyll, total soluble sugars and proline contents

Experiment - II

Seedlings (15 DAG) uprooted gently and thoroughly cleaned were treated for 6 h. with 1mM conc. of two osmoprotectants solutions i) proline and ii) trehalose. After incubation period treated seedlings were removed and were kept for 72 h in 100, 150 and 200mM NaCl solution. For control no treatment of osmoprotectans was given. After 72 h seedlings were washed thoroughly with tap water followed by distilled water wash and were analyzed for total chlorophyll, total soluble sugars and proline contents

Estimation of chlorophyll

Total chlorophyll in leaves of rice seedlings was determined as per the method described by Hiscox and Israelstam (1979). 10 ml of dimethyl sulphoxide (DMSO) was added to a 50 mg of finely cut rice leaf, in a test tube. The tubes were incubated at 65°C for three h. After incubation the tubes were cooled down at room temperature and the chlorophyll extracted was measured at 663 and 645 nm in spectrophotometer. The amount of chlorophyll a, b and total chlorophyll present in the sample were calculated using the following formula.

Chlorophyll a (mg/g fresh tissue) = 2.7(O.D at 663) - 2.69 (O.D. at 645)

Chlorophyll b (mg/g fresh tissue) = 22.9 (O.D at 645) - 4.68 (O.D at 663)

Total chlorophyll (mg/g fresh tissue) = 22.2 (O.D at 645) + 8.02 (O.D at 663)

Total soluble sugars

Total soluble sugars from the rice seedlings were determined by phenol sulphuric acid method as described by Dubois *et al*. (1956). Hundred milligram of the fresh leaf sample was extracted in 25ml of 80 percent ethanol. 5 ml of extract was evaporated to dryness on water bath and residues were dissolved in 25 ml of hot distilled water. One ml of diluted sample was pipetted in 30 ml test tube and to this; 1 ml of 5 percent freshly distilled phenol solution followed by direct addition of 5 ml of concentrated sulphuric acid was added. The contents in the test tubes were shaken and placed on an ice bath for 20 min. Intensity of red color developed was recorded at 490 nm in spectrophotometer. In a similar way 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard glucose solution containing (20-100 µg glucose) was pipette out into a series of test tubes. The volume in each test tube was made up to 1 ml with distilled water. In a blank 1 ml of distilled water taken. Sugar content was determined using following calculation formula,

Total soluble sugar (g/100 g fresh wt.) = Sample O.D x Graph factor x Dilution factor

Estimation of Proline

The proline content from seedlings was estimated as per the method described by Malik and Singh (1980).Rice seedlings (200 mg) were homogenized with 5 ml of one percent sulphosalicylic acid. The crude homogenate was centrifuged at 2000 rpm for 15 min. Volume of clear supernatant collected was adjusted to 5 ml with distilled water. To this, five ml of glacial acetic acid and 1 ml of acid Ninhydrin was added and mixed well. The tubes were placed on boiling water bath for one h. The mixture was extracted with addition of toluene. The absorbance of toluene layer was recorded at 520 nm using spectrophotometer. Blank was run simultaneously with 5 ml of one percent sulphosalicylic acid. Standard curve of proline was prepared in the range of 10-50 μg of proline. The amount of proline in the sample was calculated as: Proline (%) = Sample O.D x Standard factor x Dilution factor.

Preparation of osmoprotectant solutions

1 mM L-Proline and 1 mM of D-Trehalose

Dissolved 115.13 mg of L-Proline in distilled water and final volume make up to one liter. Dissolved 378.33 mg of D-Trehalose in distilled water and final volume make up to one liter.

RESULTS AND DISCUSSION

For first part of research, seedlings (15 Days after germination) of selected varieties were treated with 100mM, 150 mM and 200 mM levels of sodium chloride solution for 24, 48 and 72 h. For control no saline solution was applied. Samples were collected at 24, 48 and 72 h for analysis of total chlorophyll, total soluble sugars and proline contents. Jaya record highest chlorophyll and total soluble sugar contents.GR-3 recorded highest proline content. Total chrophyll, total soluble sugars and proline contents increased with salinity levels in all the varieties. Results were found to be significant (Table 1.) These results are also in agreement with the findings of Pushpam and Sree Rangasamy (2000)

Under second par of research rice seedlings (15 DAG) were treated with two osmoprotectants separately with 1 mM proline and trehalose for 6 h. Treated seedlings were further retreated with NaCl saline solution for 72 h.Again fifteen days old seedlings of rice were exposed to 0,100,150 and 200 mM NaCl solutions for 72 h. Changes in biochemical parameters were studied in the seedlings and results for the same have been reported hereunder. All biochemical parameters studied were constantly increased with salinity stress. Nearly 15 to 20 fold increase in all biochemical attributes observed upon osmoprotectant treatments than none treated seedlings. Proline treated stressed rice seedlings showed high proline content than treahalose treated as well as none treated seedlings. In trehalose treated seedlings high levels of total soluble sugars were observed than proline treated seedlings.

Effect of trehalose on biochemical attributes of rice with salinity

Chlorophyll content:

Trehalose is a non-reducing disaccharide which serves as a potential osmoprotactant during stressful conditions for recovery. Under salinity stress one visible symptom of ion accumulation in leaves is a concomitant loss of chlorophyll (Yeo and Flowers, 1983) indicating some form of disruption of the chloroplasts. After treatment with trehalose, no loss of chlorophyll was observed and seedling remained green without wilting and drooping. A drastic increase in chlorophyll activity was observed in rice upon trahalose treatment during salinity stress. Among the four varieties studied, Jaya recorded maximum chlorophyll content (21.60 mg 100 g^{-1}) followed by Dandi (20.96 mg 100 g^{-1}), whereas minimum was observed in GR-3 (13.14 mg 100 g^{-1}). Chlorophyll content was increased towards salinity levels. The varieties and salinity levels were found to be significant in all the varieties. Among the different salinity levels, 200 mM NaCl level showed maximum chlorophyll content (23.26 mg 100 g^{-1}) however, the minimum chlorophyll content was observed in control (14.05 mg 100 g^{-1}).When compared to none treated seedlings, the chlorophyll content was much higher in all the varieties

Total soluble sugars (TSS)

When compared to proline treatment as well as in control seedlings total soluble sugar content was rapidly increased by trehalose treatment Data presented in Table 1 suggested a marginal increase in the TSS content in tolerant varieties, but more in sensitive variety GR-3. These results depicted that the effect of Osmoprotectants was more in sensitive varieties than tolerant varieties. Among the four varieties, Jaya recorded maximum TSS content (10.19 g 100 g^{-1}). Whereas minimum TSS was observed in GR-3 (7.33 g 100 g^{-1}). Varieties CSR-27 and Dandi both are at par with each other. Treatment and varieties as well as interaction effects were significant. After treatment with treahalose it will enhances the levels of sugars. Garcia *et al* (1997)

Proline

Accumulation of proline was more in case of GR-3 (2.69 mg g^{-1}) whereas minimum content of proline was recorded in CSR-27 (1.11 mg g^{-1}). The varieties and treatment effects as well as interaction effects were significant. (Table 2)

Effect of proline on biochemical attributes of rice with salinity

Proline is also a potent osmoprotant act as inorganic nitrogen serves during stress conditions.

Chlorophyll

The similar pattern of results were observed in chlorophyll content with that of trahalose treatment but the increment was not much than trehalose treatment .Among four varieties, Jaya recorded the maximum chlorophyll content (16.46 mg 100 g^{-1}) followed by CSR-27 (15.13 mg 100 g^{-1}) (Table 2) and minimum content of chlorophyll was observed in GR-3 (11.52 mg 100 g^{-1}) which was at par with Dandi (11.77 mg 100 g^{-1}).The chlorophyll content increased with salinity in all the varieties, the treatment and varieties as well as interaction between Variety x Salinity level was significant .(Table 3)

Total soluble sugars (TSS)

When compared to trahalose treatment as well as in control seedlings total soluble sugar content was rapidly increased by trehalose treatment. The genotype Jaya recorded the maximum TSS content (10.19 g 100 g^{-1}) whereas the minimum content of TSS was observed in GR-3 (7.33 g 100 g^{-1}). The increase in TSS was more in tolerant varieties than sensitive varieties. Among salinity levels, 200 mM recorded the maximum TSS content (9.85 g 100 g^{-1}). But after trehalose treatment, increase in TSS content was also higher in sensitive variety GR-3 than proline treatment

Proline

Results revealed that the amount of proline is nearly more than twice after proline treatment to the salinized rice seedlings. The increase was more in sensitive variety GR-3 than tolerant varieties *viz.*, Jaya, Dandi, and CSR-27. Among four varieties studied, GR-3 recorded maximum proline content (5.09 mg g^{-1}) and minimum of proline was noticed in Dandi (4.22 mg g^{-1}), (Table 1).Proline content increased with salinity levels, 200 mM NaCl level showed highest proline content was (4.55 mg g^{-1}) than the control (3.50 mg g^{-1})

From this results, it can be concluded that all the biochemical parameters studied showed positive response with osmoprotectant treatments with none treated (Table 1). Salinized rice seedlings showed poor performance in biochemical attributes than osmoprotectant treated salinized rice seedlings. This investigation is also in agreement with Garcia *et al. (1997)*. From this research, it is noticed that trehalose protects rice seedlings better than proline. Tehalose promotes leaf growth nearly double than proline. Among both osmoprotectants studied (Proline, Trehalose) trehalose served as a potential osmoprotetrant

Table 1: Changes in biochemical attributes in rice seedlings salt stress (with out osmoprotectants treatment)

	Control (S_1)	100 mM (S_2)	150 mM (S_3)	200 mM (S_4)	Varietal mean
	Chlorophyll (mg 100 g^{-1})				
V_1 (Jaya)	2.69	4.28	5.88	2.05	3.53
V_2 (	2.71	3.16	3.77	2.27	2.98
V_3	2.45	3.07	3.91	1.99	2.85
V_4	3.10	3.89	4.84	2.28	3.75
Treatment mean	2.73	3.61	4.60	2.15	
	V		S		V x S
S.Em.	0.07		0.07		0.14
C.D.	0.20		0.20		0.41
C.V. %	7.43				
	Total Soluble Sugar (g 100 g^{-1})				
V_1	2.53	0.88	3.05	2.83	2.32
V_2	1.79	2.03	2.42	2.77	2.02
V_3	1.45	1.53	2.16	2.93	2.25
V_4	1.22	1.96	2.34	1.23	1.60
Treatment mean	1.75	1.60	2.49	2.44	
	V		S		V x S
S.Em.	0.04		0.05		0.09
C.D.	0.16		0.16		0.27
C.V. %	6.45				
	Proline (mg g^{-1})				
V_1	1.14	1.87	2.21	2.57	1.93

V_2	0.73	1.16	1.56	1.68	1.28
V_3	0.59	0.76	0.97	1.29	0.91
V_4	0.66	1.99	2.59	2.97	2.06
Treatment mean	0.78	1.43	1.83	2.13	
	V		S		V x S
S.Em.	0.03		0.04		0.07
C.D.	0.10		0.10		0.20
C.V. %	7.90				

Table 2: Changes in biochemical attributes in rice seedlings upon trehalose treatment

	Control (S_1)	100 mM (S_2)	150 mM (S_3)	200 mM (S_4)	Varietal mean
	Chlorophyll (mg 100 g^{-1})				
V_1	17.56	18.14	22.56	28.13	21.60
V_2	15.44	19.14	21.84	27.41	20.96
V_3	16.06	17.44	18.18	21.32	18.25
V_4	7.15	14.04	15.21	16.17	13.14
Treatment mean	14.05	17.19	19.45	23.26	
	V		S		V x S
S.Em.	0.12		0.12		0.24
C.D.	0.36		0.36		0.72
C.V. %	2.30				
	Total Soluble Sugar (g 100 g^{-1})				
V_1	9.72	10.39	10.58	10.07	10.19
V_2	9.17	9.46	9.89	10.28	9.70
V_3	8.83	9.02	9.70	10.70	9.56
V_4	6.09	7.10	7.79	8.35	7.33
Treatment mean	8.45	8.99	9.49	9.85	
	V		S		V x S
S.Em.	0.05		0.05		0.10
C.D.	0.15		0.15		0.30
C.V. %	1.98				
	Proline (mg g^{-1})				
V_1	1.14	1.86	2.16	2.59	1.94
V_2	1.65	1.34	1.63	1.88	1.63
V_3	0.91	1.02	1.19	1.33	1.11
V_4	2.20	2.67	2.83	3.03	2.69
Treatment mean	1.48	1.72	1.95	2.21	
	V		S		V x S
S.Em.	0.03		0.03		0.06
C.D.	0.09		0.09		0.18
C.V. %	5.81				

Table 3: Changes in biochemical attributes in rice seedlings upon proline treatment

	Control (S_1)	100 mM (S_2)	150 mM (S_3)	200 mM (S_4)	Varietal mean
	Chlorophyll (mg 100 g^{-1})				
V_1	9.06	13.44	19.16	24.16	16.46
V_2	7.74	10.59	12.27	16.47	11.77
V_3	7.16	10.06	18.08	25.21	15.13
V_4	5.82	10.40	12.99	16.88	11.52
Treatment mean	7.45	11.12	15.63	20.68	
	V		S		V x S
S.Em.	0.19		0.19		0.38
C.D.	0.54		0.54		1.08
C.V. %	4.73				
	Total soluble sugar (g 100 g^{-1})				
V_1	4.67	5.90	7.53	7.24	6.34
V_2	3.42	5.04	5.74	6.68	5.22

V_3	4.37	4.60	4.69	5.53	4.80
V_4	3.35	3.45	3.85	2.36	3.25
Treatment mean	3.95	4.75	5.45	5.45	
	V		S	V x S	
S.Em.	0.05		0.05	0.10	
C.D.	0.14		0.14	0.28	
C.V. %	3.29				
	Proline (mg g^{-1})				
V_1	3.35	4.24	4.51	4.76	4.22
V_2	3.10	3.55	3.97	4.27	3.72
V_3	2.95	3.24	3.32	3.63	3.29
V_4	4.58	4.95	5.10	5.54	5.04
Treatment mean	3.50	4.00	4.23	4.55	
	V		S	V x S	
S.Em.	0.05		0.05	0.10	
C.D.	0.13		0.13	0.26	
C.V. %	3.81				

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