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Differential Response of Brinjal (*Solanum melongena* Linn.) Varieties to Salinity Stress in Relation to Seed Germination and Osmolytes Accumulation

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ABSTRACT

The effect of NaCl stress was studied in nine commercial hybrid varieties of brinjal under various levels (0 – 200 mM) of salinity (NaCl). NaCl stress decreased germination percentage, shoot length and root length and biomass production in all the nine varieties. Among the nine varieties maximum reduction in germination, seedling growth and biomass production was observed in MEBH 10 and minimum in MHBJ 112. Therefore, for further study MEBH 10 was considered as salt sensitive and MHBJ 112 was considered as salt tolerant varieties. Increased NaCl stress decreased chlorophyll content, and increased malondialdehyde level (MDA; lipid peroxidation), free proline, glycine betaine (GB) and total soluble sugars (TSS) content in both varieties. The magnitude of increase in accumulation of MDA, free proline, GB and TSS was highest in variety MHBJ 112 than MEBH 10. The MDA content was drastically decreased at 200 mM level of NaCl in both the varieties. The decreased in germination percentage, growth of the seedlings and increased osmolyte accumulation (proline, GB and TSS) showed that MHBJ 112 is salt tolerant and MEBH 10 is salt sensitive variety.

Keywords: growth, NaCl stress, proline, glycine betaine, total soluble sugars

INTRODUCTION

Salinity and drought are the major abiotic stresses in the world especially in arid and semi-arid regions and can severely limits the plant growth and productivity of the economically important crop plants. Salinity affects nearly 20% of the world's cultivated area and about half of the world's total irrigated lands (Zhu 2001; FAO 2008). 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 (Sahi *et al*. 2006). Interaction of salt with the plants may depend upon salt type, concentration and the genotype. Therefore, screening for tolerant genotypes that could with stand extreme environmental conditions such as salinity and drought will ensure future crop production (Jogeswar *et al*. 2006).

Field evaluation of salt tolerant species is difficult due to complex environmental conditions and interactions which are not easily controlled and differential sensitivity to salt during the various stages of a plant's life cycle (Flowers 2004). Salt stress affects almost every aspect of plant physiology at both whole plant and cellular levels through osmotic and ionic stress (Hasegawa *et al*. 2000; Murphy and Durako 2003). Salinity is detrimental to the various processes of crops such as metabolism of plant cell, seed germination, seedling growth and vigour, vegetative growth, flowering and fruit setting, leading to severe crop damage; which results in decreased crop yield (Greenway and Munns 1980; Fowler 1991; Vaidyanathan *et al*. 2003; Sairam and Tyagi 2004). Successful seedling establishment depends on the ability of the species to germinate and grow vigorously when soil moisture and osmotic potentials decrease (Roundy 1987; Welbaum *et al*. 1990). Germination and seedling characteristics are the most useful criteria for selecting salt tolerance in plants (Boubaker 1996). Salt stress causes inhibition of growth and development, reduction in photosynthesis, respiration, and protein synthesis (Levine *et al*. 1990; Sairam *et al*. 2002).

Chlorophyll loss, lipid peroxidation in terms of MDA content and electrolyte leakage are considered to be indicators of oxidative damage (Dhindsa and Mathowe 1981; Wise and Naylor 1987). Accumulation of proline is widely accepted as a marker for salt/drought stress (Storey and Wyn-Jones 1975; Naik and Joshi 1983; Kavi Kishor *et al*. 2005), which protects the proteins against denaturation and also act as osmotic balancing agents (Chadalavada *et al*. 1994; Sivakumar *et al*. 2000). Occurrence of quaternary ammonium compound GB in response to salinity stress has been reported in barley, beet and some members belonging to family chenopodiaceae (Stewart and Lee 1974).

Brinjal or eggplant (*Solanum melongena* Linn.), is a very important common vegetable in India. It is often described as a poor person's vegetable because it is popular amongst small-scale farmers and low income consumers. It is featured in the dishes of virtually every household in India, regardless of food preferences, income levels and social status (Choudhary and Gaur 2009). Low in calories and high in nutrition, the vegetable has very high water content and is a very good source of fiber, calcium, phosphorus, folate, and vitamins B and C (Collonnier *et al*. 2001). It is also used in *ayurvedic* medicine for curing diabetes, hypertension and obesity. In addition, dried brinjal shoots are used as fuel in rural areas (Choudhary and Gaur 2009).

Contradictory literature exists on eggplant tolerance to soil salinity; some classified the eggplant as a moderately sensitive vegetable crop (Heuer *et al*. 1986; Savvas and Lenz 1996), whereas Unlukara *et al*. (2010) reported that the eggplant is sensitive to water stress caused by salinity. Akinci *et al*. (2004) also reported that, the eggplant is affected negatively by increasing salt at the germination and seedling stages. The salt tolerance of the different varieties of the eggplants is varied from variety to variety (Akinci *et al*. 2004).

Therefore, in the present investigation, we have studied the differential response of brinjal varieties to NaCl stress in

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terms of physiological and biochemical parameters including seed germination and growth, chlorophyll contents, lipid peroxidation, proline, GB and TSS.

MATERIALS AND METHODS

Plant material

The popular hybrid varieties of brinjal in Pune region, namely MHB-4, MEBH-10 and MHBJ-112 (Mahyco, Mumbai), Ajay, Utkarsha, ARBH-1095 (Ankur Seeds Pvt. Ltd., Nagpur), Manjari (Monsanto Holdings Pvt. Ltd., Mumbai), Manju (Syngenta India Ltd., Pune) and Tapiraja (Amar Seeds Pvt. Ltd., Pune) were purchased from the Market Yard, Pune (Maharashtra, India) and used for the experimentation.

Salinity treatment and culture conditions

Seeds of all varieties were surface sterilized with 0.1% (w/v) mercuric chloride for 2 min and then washed five times with sterile distilled water (SDW). Twenty five seeds of each variety were sown in a sterile Petri dish (10 cm diameter, Axygen, India) containing two layer of germination paper (1 mm thick, Modern Paper Ltd., India). Initially the germination paper was moistened with 10 ml of distilled water considered as control and different concentrations of NaCl solutions (i.e. 50, 100, 150 and 200 mM). Every day 2 ml of NaCl solutions (treatment) and distilled water (control) was applied to respective Petri dish and all the observations were recorded on the 14th day after sowing (DAS). The Petri plates were maintained at room temperature in the dark.

Growth analysis

The seeds in which 0.5 mm or more radical growth occur were counted as germinated seeds. The primary data on seed germination was collected daily (maximum up to 14 days). The final germination percentage (FGP) was calculated from the total seeds that germinated on the 14th DAS. Root length and shoot length of the seedlings were recorded and root to shoot ratio was calculated. The fresh weight (FW) of seedlings was recorded and the seedlings were dried in an oven at 60^{o}C until constant weight and then dry weight (DW) of seedlings were recorded. Moisture content of seedlings was calculated using the formula:

FW - DW/FW × 100

All observations were recorded on the 14th DAS.

Biochemical analysis

***Determination of chlorophyll*:** Chlorophyll contents were estimated by extracting 100 mg of the seedling samples in 5 ml 80% (v/v) acetone (Qualigens, Mumbai, India). Then the samples were centrifuged at 5000 rpm for 5 min and the absorbance of supernatant was recorded at 645, 652 and 663 nm on UV-VIS spectrophotometer (Shimadzu – 1601, Japan). Chlorophyll contents were calculated as per standard method (Arnon 1949).

***Determination of lipid peroxidation*:** The level of lipid peroxidetion was measured in terms of MDA contents (Heath and Packer 1968). Fresh samples (400 mg) were homogenized in 8 ml of 0.25% thiobarbituric acid (TBA; Hi Media, Mumbai, India) in 10% trichloro-acetic acid (TCA; Hi Media, Mumbai, India). Then the mixture was heated at 95°C in water bath for 30 min and quickly cools on ice bath. This was followed by centrifugation at 10,000 rpm for 10 min to remove suspended turbidity and then the absorbance was recorded at 532 nm by keeping the 0.25% TBA in 10% TCA as blank. The value for non-specific absorption at 600 nm was subtracted and the MDA content was calculated using its absorption coefficient of 155 $mmol^{-1}$ cm^{-1}.

***Determination of proline*:** The proline content was determined from the seedling tissue by following the method of Bates *et al*. (1973). Samples (500 mg) were homogenized in 10 ml of 3% (w/v) sulphosalicylic acid (Merck, Mumbai, India) using mortar and pestle followed by centrifugation at 10,000 rpm for 10 min. To the 2 ml of supernatant equal volume of glacial acetic acid (Qualigens, Mumbai, India) and acid ninhydrin (Hi Media, Mumbai, India) reagent were added and the reaction mixture was boiled in water bath at 100°C for 1 h. Then the reaction was terminated in an ice bath following by addition of 4 ml toluene (Qualigens, Mumbai, India). After thorough mixing, the chromophore containing toluene was separated and absorbance was recorded at 520 nm in UV-VIS spectrophotometer against toluene blank. Concentration of proline was estimated by referring to a standard curve of proline.

***Determination of glycine betaine*:** GB estimation was done according the method as described by Grive and Grattan (1983). The plant material submerges into liquid nitrogen and ground the samples using mortar and pestle. Finely powdered plant material (500 mg) was mechanically shaken with 20 ml of deionized water for 16 h at 25°C. Then the samples were filtered and the filtrate was store in freezer. Then the thawed extracts were diluted 1:1 with 2 N H_2SO_4 (Qualigens, Mumbai, India). From this 500 µl was measured into test tube and cooled in ice water for 1 hour, then to this 200 µl of cold KI-I_2 reagent was added and the mixture was gently mixed. The samples were stored at 4°C for 16 h. Then the samples were centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was carefully aspirated as the solubility of the periodite complexes in the acid reaction mixture increases markedly with temperature. The periodite crystals were dissolved in 9 ml of 1,2-dichloro ethane (Qualigens, Mumbai, India). After 2.0-2.5 h the absorbance was measured at 365 nm with UV-VIS spectrophotometer against the 1, 2-dichloroethane blank. Reference standards of GB (50 – 200 mg ml^{-1}) were prepared in 2 N H_2SO_4 and the procedure for sample estimation was followed.

***Total soluble sugars (TSS) content*:** TSS was estimated as per the anthrone method (Watanabe *et al*. 2000) with some modifications (Lokhande *et al*. 2010). About 200 mg of samples were homogenized with ice-cold 80% ethanol in a mortar and pestle. The homogenate was centrifuged at 5,000 rpm for 10 min at 4°C, and then the final volume was adjusted to 10 ml with 80% ethanol. From this 1 ml of supernatant was reacted with 3 ml of freshly prepared anthrone reagent. Then the reaction mixture was incubated for 10 min at 100°C in a hot water bath. The reaction was terminated by quick cooling in an ice bath and allowed to cool at room temperature. The optical density was measured spectrophotometrically at 620 nm. A standard curve was prepared using D-glucose (Hi Media, Mumbai, India); the TSS was calculated and expressed as mg g^{-1} FW.

Statistical analysis

Each Petri dish was considered as replicate and all of the treatments were repeated three times and data are expressed as mean ± standard error (SE). Data were analyzed by analysis of variance (ANOVA) to detect significant differences between means. Means differing significantly were compared using Duncan's multiple range test (DMRT) at the 5% probability level using the computer software program SPSS (version 9.1).

RESULTS AND DISCUSSION

The responses of plants to salt stress at germination level is particularly important for elucidating the mechanisms of salt resistance or sensitivity in plants, their survival and successful crop production (Mayer and Poljakoff-Mayber 1963; Almansouri *et al*. 2001). The seed germination decreased with increasing salt concentration from 50-200 mM NaCl. However, the effect of salt stress on seed germination varied between the varieties (**Table 1**). In the control, 100% seed germination was observed in all the varieties. As the NaCl concentration goes on increasing noticeable reduction in germination percentage was observed in all varieties. Variety MHBJ 112 was comparably least affected at higher salt concentrations. About 80.0 ± 0.0% germination was observed in variety MHBJ 112 at 200 mM NaCl and in variety MEBH 10, it was only 18.7 ± 2.7% at higher 200 mM NaCl

Table 1 Effect of NaCl stress on germination percentage, root length, shoot length and root/shoot ratio in brinjal varieties.

Variety	NaCl stress	Germination Percentage (%)	Root length (mm)	Shoot length (mm)	Root/Shoot Ratio
MEBH 10	0 (Control)	100.0 ± 0.0 a	51.4 ± 3.9 ab	62.0 ± 4.0 a	0.8
	50 mM	92.0 ± 2.3 b	47.8 ± 3.3 b	50.8 ± 1.8 b	0.9
	100 mM	82.7 ± 1.3 c	50.0 ± 3.5 ab	40.2 ± 1.8 c	1.3
	150 mM	65.3 ± 3.5 d	58.4 ± 2.0 a	32.8 ± 1.2 d	1.8
	200 mM	18.7 ± 2.7 e	29.8 ± 1.6 c	09.0 ± 0.9 e	3.5
MHBJ 112	0 (Control)	100.0 ± 0.0 a	53.4 ± 3.5 ab	63.2 ± 1.5 a	0.8
	50 mM	97.3 ± 1.3 b	55.0 ± 2.2 a	44.6 ± 1.4 b	1.3
	100 mM	94.7 ± 1.3 c	53.4 ± 5.5 ab	27.8 ± 2.0 c	1.9
	150 mM	92.0 ± 0.0 d	44.0 ± 2.9 b	18.4 ± 2.1 d	2.5
	200 mM	80.0 ± 0.0 e	19.0 ± 1.7 c	07.8 ± 0.7 e	2.5
MHB 4	0 (Control)	100.0 ± 0.0 a	46.8 ± 3.9 cd	57.6 ± 3.3 a	0.8
	50 mM	94.7 ± 1.3 ab	72.4 ± 5.8 ab	55.8 ± 1.9 a	1.3
	100 mM	93.3 ± 2.7 ab	81.4 ± 2.3 a	39.0 ± 1.3 b	2.1
	150 mM	90.7 ± 1.3 b	62.4 ± 2.5 bc	27.6 ± 1.1 c	2.3
	200 mM	52.0 ± 4.0 c	38.6 ± 6.0 d	11.6 ± 2.2 d	3.4
Ajay	0 (Control)	100.0 ± 0.0 a	44.0 ± 3.8 a	58.4 ± 3.8 a	0.8
	50 mM	94.7 ± 2.3 b	44.6 ± 5.3 a	53.2 ± 6.5 a	0.8
	100 mM	86.7 ± 2.3 c	49.8 ± 3.5 a	33.0 ± 4.7 b	1.2
	150 mM	80.0 ± 4.0 d	29.6 ± 2.1 b	24.2 ± 4.5 c	1.4
	200 mM	25.3 ± 2.3 e	25.6 ± 2.9 b	18.8 ± 0.4 c	1.5
Utkarsha	0 (Control)	100.0 ± 0.0 a	43.4 ± 1.7 b	50.4 ± 1.1 a	0.9
	50 mM	94.7 ± 1.1 ab	56.8 ± 1.1 a	51.2 ± 2.7 a	1.1
	100 mM	90.7 ± 1.1 b	54.4 ± 2.7 a	36.0 ± 4.8 b	1.3
	150 mM	54.7 ± 3.9 c	19.6 ± 1.7 c	15.0 ± 1.1 c	1.5
	200 mM	22.7 ± 1.1 d	17.4 ± 1.0 c	10.4 ± 1.0 c	1.7
ARBH-1095	0 (Control)	100.0 ± 0.0 a	45.0 ± 2.6 b	64.0 ± 2.5 a	0.7
	50 mM	93.3 ± 1.3 b	52.0 ± 3.8 a	45.6 ± 2.8 b	1.1
	100 mM	86.7 ± 2.7 c	57.8 ± 1.9 a	40.4 ± 2.1 b	1.4
	150 mM	42.7 ± 2.7 d	31.8 ± 1.5 c	14.2 ± 1.3 c	2.3
	200 mM	21.3 ± 1.3 e	26.6 ± 0.9 c	08.2 ± 0.6 d	3.3
Manjari	0 (Control)	100.0 ± 0.0 a	52.0 ± 6.0 abc	59.0 ± 2.5 a	0.9
	50 mM	96.0 ± 2.3 ab	62.0 ± 5.3 a	47.0 ± 1.9 b	1.3
	100 mM	89.3 ± 2.7 bc	57.4 ± 2.1 ab	38.2 ± 3.0 c	1.5
	150 mM	81.3 ± 4.8 c	47.8 ± 1.7 bc	17.4 ± 1.0 d	2.8
	200 mM	25.3 ± 1.3d	43.2 ± 1.6 c	10.0 ± 0.7 e	4.4
Manju	0 (Control)	100.0 ± 0.0 a	54.0 ± 1.9 b	59.4 ± 2.0 a	0.9
	50 mM	94.7 ± 3.5 ab	44.0 ± 2.8 cd	40.4 ± 0.9 b	1.1
	100 mM	90.7 ± 3.5 b	72.2 ± 5.4 a	36.4 ± 4.7 b	2.1
	150 mM	78.7 ± 2.7 c	48.2 ± 5.1 bc	20.4 ± 0.9 c	2.4
	200 mM	24.0 ± 2.3 d	37.8 ± 1.7 d	10.6 ± 0.4 d	3.6
Tapiraja	0 (Control)	100.0 ± 0.0 a	51.8 ± 1.9 b	63.8 ± 4.1 a	0.8
	50 mM	90.7 ± 1.3 b	69.4 ± 8.7 a	38.0 ± 1.4 b	1.8
	100 mM	81.3 ± 2.7 c	50.4 ± 0.9 b	31.8 ± 2.0 b	1.6
	150 mM	42.7 ± 2.7 d	47.2 ± 1.4 b	14.4 ± 1.5 c	3.5
	200 mM	21.3 ± 1.3 e	33.4 ± 1.9 c	07.0 ± 0.7 d	5.1

Data presented in the table are mean ± SE (standard error) scored at 14 DAS from 3 Petri dishes per treatment and repeated thrice. Mean followed by same letters within columns are not significantly different at $P \leq 0.05$ level by Duncan's multiple range test. DMRT was applied to each variety separately.

(**Table 1**). Variety MEBH 10 was most affected at higher salt concentration as compare to the other varieties. While other varieties, MHB 4, Ajay, Utkarsha, ARBH-1095, Manjari, Manju and Tapiraja showed moderate response for seed germination at higher salt concentration.

The increase in salinity decreased the shoot and root lengths considerably in all the varieties, with more reduction in shoot length than the root length. Therefore, the root/shoot ratio was goes on increasing as the salinity increases in all the varieties. The higher root/shoot ratio (5.1) was found in the variety Tapiraja at higher salt concentration (200 mM NaCl) and lower (1.5) was found in the variety Ajay (**Table 1**). A negative correlation between biomass production and salinity stress was evident from the results obtained (**Table 2**) as seedling fresh weight, seedling dry weight and moisture content gradually decrease in all the varieties with increase in NaCl concentration.

The germination percentage was drastically decline at 200 mM NaCl in variety MEBH 10 than the variety MHBJ 112. The growth parameters like root length and shoot length was also reduced with increasing concentration of NaCl in all the varieties of brinjal. The shoot length was more inhibited by the increasing concentration of NaCl than root length. Therefore, increase in the root/shoot ratio was observed in all the varieties with increasing NaCl concentration. Similar results were recorded by Akini *et al*. (2004) in different varieties of brinjal. The negative effect of NaCl on the germination stage characteristics were in accord with the findings of Jones *et al*. (1989) for cucumber, Coons *et al*. (1990) for lettuce, Goertz and Coons (1991) for beans, Kumar *et al*. (2007) for rice and Patil *et al*. (2010) for niger. Higher salt concentration reduces the water potential which hinders water absorption by germinating seeds and this results in a reduction of germination percentage (Mass and Neiman 1978). It is assumed that germination rate and the final seed germination decrease with the decrease of the water movement into the seeds during imbibitions (Hadas 1977). Salinity stress can affect seed germination through osmotic effects (Welbaum *et al*. 1990). Salt induced inhibition of seed germination could be attributed to osmotic stress or to specific ion toxicity (Huang and Redmann 1995).

The results of the germination percentage, growth parameters (root length, shoot length, root/shoot ratio) and biomass production (seedling fresh weight and seedling dry weight) indicates that MHBJ 112 was less affected by salinity stress, while these parameters were markedly affected in MEBH 10. The other varieties (MHB 4, Ajay, Utkarsha, ARBH-1095, Manjari, Manju and Tapiraja) showed inter-

Table 2 Effect of NaCl stress on fresh, dry weights and moisture content of seedlings in brinjal varieties.

Variety	NaCl stress	Fresh weight (mg)	Dry weight (mg)	Moisture content (%)
MEBH 10	0 (Control)	51.2 ± 3.6 ab	3.0 ± 0.6 ab	94.1
	50 mM	48.2 ± 2.4 a	3.0 ± 0.4 a	93.7
	100 mM	33.8 ± 2.9 bc	2.6 ± 0.6 ab	92.3
	150 mM	27.2 ± 3.9 c	2.2 ± 0.7 ab	91.9
	200 mM	14.0 ± 1.1 d	1.8 ± 0.6 b	87.1
MHBJ 112	0 (Control)	52.0 ± 3.2 a	4.4 ± 1.0 a	91.5
	50 mM	51.8 ± 4.6 a	4.4 ± 0.2 a	91.5
	100 mM	40.0 ± 4.2 b	3.2 ± 0.4 a	92.0
	150 mM	31.8 ± 1.2 b	3.8 ± 0.6 a	88.0
	200 mM	19.8 ± 1.1 c	3.0 ± 0.5 a	84.0
MHB 4	0 (Control)	51.8 ± 2.5 a	3.4 ± 0.2 a	93.4
	50 mM	48.2 ± 4.9 a	3.0 ± 0.5 a	93.7
	100 mM	39.0 ± 1.9 a	2.6 ± 0.4 a	93.3
	150 mM	28.4 ± 2.6 b	2.2 ± 0.4 a	92.2
	200 mM	16.0 ± 1.6 b	1.9 ± 0.2 a	88.1
Ajay	0 (Control)	57.6 ± 6.8 a	2.4 ± 0.7 b	96.0
	50 mM	47.7 ± 5.5 ab	3.3 ± 0.3 ab	92.7
	100 mM	44.6 ± 2.3 b	3.5 ± 0.4 ab	92.0
	150 mM	20.9 ± 1.2 c	3.6 ± 0.1 a	82.4
	200 mM	13.6 ± 0.5 d	4.0 ± 0.1 a	70.5
Utkarsha	0 (Control)	42.0 ± 3.4 a	2.3 ± 0.4 c	94.7
	50 mM	44.0 ± 3.5 a	2.7 ± 0.5 bc	94.0
	100 mM	32.7 ± 2.5 b	3.2 ± 0.3 abc	90.1
	150 mM	13.7 ± 0.6 c	3.7 ± 0.4 ab	73.3
	200 mM	09.8 ± 0.4 c	3.9 ± 0.1 a	59.8
ARBH-1095	0 (Control)	44.0 ± 4.0 a	4.8 ± 0.1 a	88.6
	50 mM	42.1 ± 2.9 a	3.4 ± 0.3 b	91.8
	100 mM	40.5 ± 2.8 a	2.8 ± 0.4 b	92.7
	150 mM	21.5 ± 2.5 b	3.0 ± 0.2 b	85.0
	200 mM	15.0 ± 1.3 b	2.6 ± 0.2 b	82.0
Manjari	0 (Control)	42.5 ± 2.7 a	2.4 ± 0.3 a	94.5
	50 mM	38.1 ± 2.2 a	3.0 ± 0.4 a	92.1
	100 mM	38.7 ± 2.8 a	3.4 ± 0.2 a	91.2
	150 mM	23.0 ± 1.9 b	3.3 ± 0.6 a	85.7
	200 mM	11.0 ± 1.0 b	3.6 ± 0.8 a	66.3
Manju	0 (Control)	54.3 ± 7.3 a	2.6 ± 0.4 a	95.1
	50 mM	39.2 ± 1.5 b	3.0 ± 0.1 a	92.3
	100 mM	30.9 ± 3.0 b	3.3 ± 0.2 a	89.2
	150 mM	16.8 ± 0.7 c	3.2 ± 0.2 a	80.8
	200 mM	14.6 ± 2.2 c	4.1 ± 0.6 a	71.5
Tapiraja	0 (Control)	49.1 ± 1.0 a	2.5 ± 0.3 b	94.9
	50 mM	48.6 ± 3.0 a	3.8 ± 0.2 a	92.1
	100 mM	26.4 ± 1.1 b	3.1 ± 0.4 ab	88.2
	150 mM	17.0 ± 0.8 c	3.6 ± 0.2 a	79.0
	200 mM	14.5 ± 0.8 c	3.3 ± 0.3 ab	77.3

Data presented in the table are mean ± SE (standard error) scored at 14 DAS from 3 Petri dishes per treatment and repeated thrice. Mean followed by same letters within columns are not significantly different at $P \leq 0.05$ level by Duncan's multiple range test. DMRT was applied to each variety separately.

mediate response to NaCl induced salinity stress at germination level. Therefore, for further salt stress experiments we used MHBJ 112 as salt tolerant and MEBH 10 as salt sensitive varieties.

Chlorophylls (Chl *a* and *b*) are main photosynthetic pigments and plays important role in photosynthesis. The changes in the amount of pigments were evaluated as the changes in photosynthetic ability of the plants. The changes in the Chl content under salt stress are used as the parameters for selection of tolerant and sensitive crop cultivars (Doganlar *et al*. 2010). Chl contents in our results were markedly reduced under NaCl stress in both the varieties. The salt sensitive variety MEBH 10 showed significantly decline in Chl *a*, *b* and total Chl contents than tolerant variety MHBJ 112 (**Table 3**). Similar observations were reported earlier and Chl contents were reduced more in salt sensitive variety than salt tolerant one. Highly significant decrease in Chl content with increasing salinity in salt sensitive rice genotype than salt tolerant genotype was described by many authors (Lutts *et al*. 1996; Misra *et al*. 1997; Kumar *et al*. 2007). Similarly decrease in Chl content with increasing salinity in salt sensitive cultivars than salt tolerant cultivars was recorded by Patil *et al*. (2010) in Niger. According to Choudhury and Choe (1997), a decline in photosynthetic rate with increased NaCl concentration may be associated with decreased in pigmentation. Our results are in agreement with this hypothesis.

The generation of reactive oxygen species is a common response to stress conditions, such as salinity and drought (Gosset *et al*. 1994; Luna *et al*. 1994). Reactive oxygen species cause membrane lipid peroxidation, reducing membrane fluidity and selectivity. Lipid peroxidation measured as MDA content is considered to be indicator of oxidative damage from stress (Dhindsa and Matowe 1981). High levels of H_2O_2 can also accelerate processes like Haber–Weiss reaction, resulting in the formation of hydroxyl radicals that can cause lipid peroxidation (Loggini *et al*. 1999). This is reflected in the greater extent of lipid peroxidation (Vaidyanathan *et al*. 2003). MDA content might be associated with the minimum oxidative damage to membrane and therefore, lower production of H_2O_2, which was found to be responsible for reduction in peroxidation of membrane lipids and better osmotic adjustment (Lokhande *et al*. 2011).

Our results on the increased MDA content under the control and salt (50 – 200 mM NaCl) suggest oxidative stress conditions. The NaCl concentration up to 150 mM showed linear increase in MDA content but there is drastic decline in the MDA content at 200 mM NaCl concentration (**Fig. 1**). The presence of strong antioxidative defense mechanism combined with physiological specialization in the plant contributes to the difference in salt tolerance capacity (Cherian and Reddy 2003). Similarly, Yasar *et al*. (2006) reported the increase in MDA content with increasing NaCl stress conditions in brinjal cultivars. The magnitude of increase in the MDA content was more in salt tolerant cultivar than the salt sensitive one. The results of the present investigation are also in agreement with the results of Yasar *et al*. (2006).

Osmolyte accumulation is frequently reported in plants exposed to salt stress, and has been correlated with plants capacity to tolerate and adapt to salinity conditions (Errabii *et al*. 2007; Slama *et al*. 2008). Proline is one of the most important osmoprotectant and widely studied osmolyte found in plants and is related with abiotic stress tolerance (Sivakumar *et al*. 2002). Proline is generally assumed to serve as a physiologically compatible solute that increases as needed to maintain favorable osmotic potential between the cell and its surroundings (Pollard and Wyn Jones 1979). Dramatic accumulation of proline due to increased synthesis and decreased degradation under a variety of stress conditions such as salt, drought and metal has been docu-

Table 3 Effect of NaCl stress on chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and total chlorophyll contents in brinjal varieties.

NaCl (mM)	Chl *a* (mg g^{-1} FW)		Chl *b* (mg g^{-1} FW)		Total chlorophyll (mg g^{-1} FW)	
	MEBH 10	MHBJ 112	MEBH 10	MHBJ 112	MEBH 10	MHBJ 112
Control	0.024 ± 0.001 a	0.027 ± 0.001 a	0.008 ± 0.001 a	0.008 ± 0.001 a	0.033 ± 0.001 a	0.041 ± 0.001 a
50	0.019 ± 0.001 b	0.021 ± 0.001 b	0.005 ± 0.001 b	0.006 ± 0.001 b	0.027 ± 0.001 b	0.034 ± 0.001 b
100	0.014 ± 0.001 c	0.015 ± 0.001 c	0.005 ± 0.001 c	0.002 ± 0.001 c	0.020 ± 0.001 c	0.028 ± 0.001 c
150	0.009 ± 0.001 d	0.013 ± 0.001 d	0.002 ± 0.001 d	0.002 ± 0.001 d	0.018 ± 0.001 d	0.018 ± 0.001 d
200	0.007 ± 0.001 e	0.009 ± 0.001 e	0.002 ± 0.001 e	0.002 ± 0.001 e	0.013 ± 0.001 e	0.015 ± 0.001 e

Data presented in the table are mean ± SE (standard error) scored at 14 DAS from 3 Petri dishes per treatment and repeated thrice. Mean followed by same letters within columns are not significantly different at $P \leq 0.05$ level by Duncan's multiple range test.

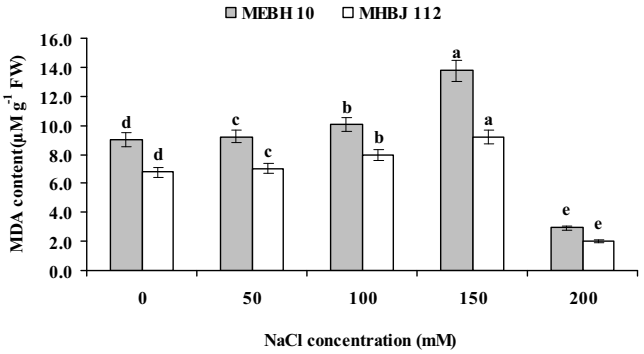

Fig. 1 Effect of different concentrations of NaCl stress on lipid peroxidation (in terms of MDA content) in brinjal varieties. Each value represents mean of three replications and vertical bars indicate SE. Data are statistically significant at $P < 0.05$. DMRT was applied to each variety separately.

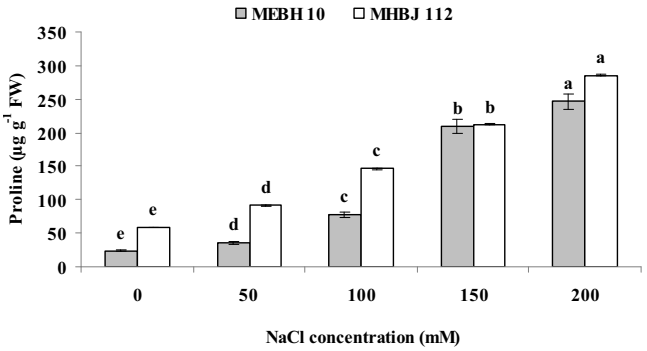

Fig. 2 Effect of different concentrations of NaCl stress on proline content in brinjal varieties. Each value represents mean of three replications and vertical bars indicate SE. Data are statistically significant at $P < 0.05$. DMRT was applied to each variety separately.

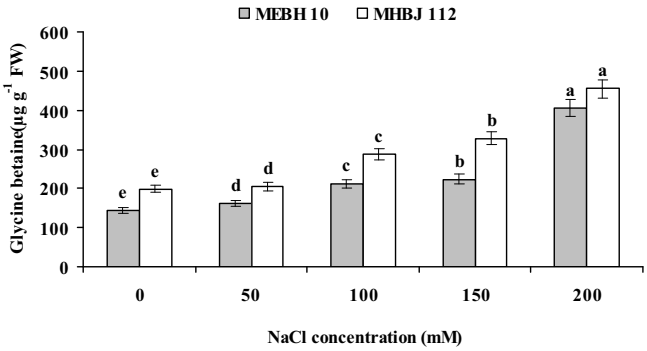

Fig. 3 Effect of different concentrations of NaCl stress on glycine betaine content in brinjal varieties. Each value represents mean of three replications and vertical bars indicate SE. Data are statistically significant at $P < 0.05$. DMRT was applied to each variety separately.

mented in many plants (Kavi Kishor *et al*. 2005), in some cases several times the sum of all other amino acids (Mansour 2000). In the present study, free proline content was significantly increased in the stressed plants over control plants at all salt treatments in both the varieties. Higher accumulation of proline was observed at 200 mM NaCl in salt tolerant variety MHBJ 112 compared to the salt sensitive variety MEBH 10 (**Fig. 2**). Similar to these results, Sairam *et al*. (2002) in wheat, Jogeshwar *et al*. (2006) in sorghum, Kumar *et al*. (2007) in rice and Patil *et al*. (2010) in Niger observed much higher proline accumulation in the salt tolerant genotype than the salt sensitive genotype.

It is well-known that salinity stress causes irregularities in nitrogen metabolism and that certain quaternary ammonium compounds (QAC), for example GB, are often ac-

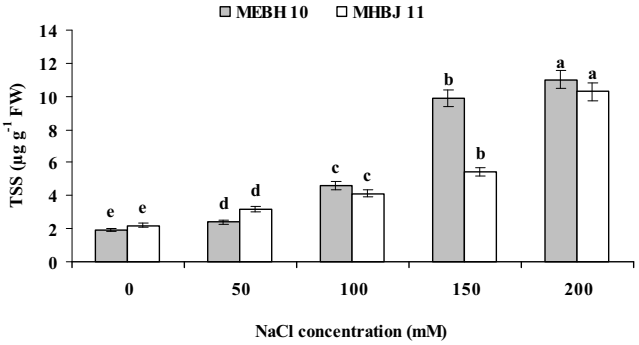

Fig. 4 Effect of different concentrations of NaCl stress on total soluble sugar content in brinjal varieties. Each value represents mean of three replications and vertical bars indicate SE. Data are statistically significant at $P < 0.05$. DMRT was applied to each variety separately.

cumulated in plant shoots (Grieve and Grattan 1983). GB is synthesized by several plant families in response to salt or osmotic stress, and serves as a compatible solute to protect proteins and membranes from the damaging effects of salts as well as functioning in cytoplasmic osmoregulation (Yancey 1994; Papageorgiou and Mutata 1995). The accumulation of this compatible solute plays a role in the adaptation of many organisms, such as bacteria (Lanfald and Strom 1986) and higher plants (Rhodes and Hanson 1993), to high salinity. In the present investigation, accumulation of glycine betaine was higher at higher NaCl dose (200 mM) as compared to the control in both the varieties irrespective of their salinity tolerance. Comparatively high accumulation of GB was observed in salt tolerant variety MHBJ 112 at 200 mM NaCl (**Fig. 3**). Similar results were recorded in salt stressed barely plants by Nakamura *et al*. (1996). Similarly, increase in accumulation of GB with increasing salinity was observed by Sumithra *et al*. (2006) in *Vigna radiata*, by Patil *et al*. (2010) in Niger.

Apart from the accumulation of proline and GB, TSS also plays an important role in maintaining the osmotic balance under stress conditions (Srivastava *et al*. 2010). In the present study, higher accumulation of total soluble sugars was increased with increasing salinity level in both the varieties (**Fig. 4**). The accumulation of TSS in salt-tolerant variety (MHBJ 112) was lower at higher NaCl concentration (200 mM) as compare to the salt sensitive variety MEBH 10 at this concentration. The increase in TSS content was found to increasing with increasing salt concentration irrespective of salt tolerant capacity of the brinjal varieties used. Salinity-induced soluble sugar accumulation has also been observed in *P. euphratica* (Watanabe *et al*. 2000; Zhang *et al*. 2004).

CONCLUSIONS

From the results of the present investigation, we can conclude that NaCl stress affected germination and seedling growth significantly. Among the 9 hybrid brinjal varieties; variety MHBJ 112 showed lesser affect of NaCl stress on germination and seedling growth, while variety MEBH 10 was highly affected by the NaCl stress. Comparably lower amount of MDA content and higher amount of osmolyte accumulation in variety MHBJ 112 suggested the salt tolerant capacity of the variety. The results obtained in the present investigation clearly indicates that the variety MHBJ 112 is comparably salt tolerant while variety MEBH 10 is found to be salt sensitive which needs improvement for the abiotic stress tolerance.

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