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Differential salt stress responses in *Solanum melongena* L. and *Solanum melongena* var. *insanum* (L.) Prain

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Abstract

Salt stress responses in *Solanum melongena* L. (cultivated brinjal) and *Solanum melongena* var. *insanum* (L.) Prain (wild brinjal) were evaluated using a set of 40 days old plants from both the genotypes. A set of plants from both genotypes were treated with 0.5% (T1), 1% (T2) and 1.5 %(T3) Nacl solution (w/v) for imposing salinity stress. Another set was maintained as control for each set of experiment. Morphological, physiological and biochemical changes were studied in both genotypes under stress and controlled conditions. Present study revealed a superior salt tolerance in *S. melongena* var. *insanum* when compared to that of *S. melongena* in terms of plant growth and development parameters as well as physiolocial and biochemical parameters evaluated. Present results suggested the use of wild S. *melongena* var. *insanum* as a reservoir of new alleles to confer stress tolerance to cultivated species of *S. melongena*.

Keywords: eggplant, differential response, *Solanum melongena*, salt tolerance

Introduction

Salt stress is believed to be the prime reason for the decreased yield of crops in the irrigated arid and semi-and regions of the world. Salt affected lands occur in practically all climatic regions, from the humid tropics to the Polar regions, and it can be found at different altitudes, from below sea level to mountains rising above 5000 meters (Singh and Chatrath, 2001). Na^+, Cl^-, HCO_3^-, K^+, Ca_2^+, Mg_2^+ and SO_4^{2-} ions usually contribute to the soil salinity. World wide more than 45 million hectares of irrigated land have been damaged by salt, and 1.5 million hectares are taken out of production each year as a result of high salinity level in the soil (Munns and Tester, 2008).

Brinjal *(Solanum melongena*) is an important vegetable in India. Solanum melongena is commonly known as eggplant or aubergine. *Solanum melongena* var. *insanum* is the wild progenitor of common eggplant (*S. melongena*) *Solanum* is the largest and most economically important genus in the family Solanaceae and comprises around 1400 species world wide (Bohs, 2005). *Solanum melongena*. L (bringal) and *Solanum melongena* var. *insanum* (wild brinjal) belongs to the Solanum subgenus *Leptostemonum*.

Brinjal is often described as poor person's vegetable because it is popular amongst small scale farmers and low income consumers (Choudhary and Gaur, 2009). In Southern India, eggplant is crowned as 'King of the vegetables' and is an important ingredient in curries, sambhars and chutney. So by considering the need, there is a large scale production of brinjal is essential. But the soil salinity is an important limiting factor for the production of brinjal in irrigated conditions. Hence development of salt tolerant varieties is very much essential in this scenario. The literature existing on the eggplant tolerance to soil salinity is very contradictory. It has been classified as moderately sensitive vegetable crop by some authors (Heuer *et. al*., 1986; Savvas and Lenz, 1996) and as sensitive by some others (Bresler *et. al*., 1982; Unlukara *et. al.*, 2010). Hence the present study has been defined with a major objective

to analyse the differential responses of wild and cultivated brinjal varieties to NaCl stress in terms of morphological, physiolocial and biochemical parameters.

Materials and methods

The experimental material for the present study comprised of common eggplant (*Solanum melongena*) and its wild progenitor *Solanum melongena* var. *insanum* (L.) Prain. The seeds of *Solanum melongena*. L were obtained from College of Agriculture, Vellayani, Thiruvananthapuram and the seeds of *Solanum melongena* var. *insanum* were collected from Parassala, Thiruvananthapuram. Healthy and uniform seeds were germinated and grown in 20L plastic bags. Almost all the seeds were germinated within 5 days and produced healthy seedlings which were grown for 40 days under natural conditions of light, humidity and temperature. Plants were watered well in morning and evening.

After a growth period of 40 days, plants were treated with 0.5 % (T1), 1% (T2) and 1.5 %(T3) NaCl solution (w/v) for imposing salinity stress. A set of plants were maintained as control for each set of experiment. Salt solutions were prepared by dissolving 0.5g, 1g and 1.5g NaCl in 100 ml distilled water respectively. For inducing salinity stress, respective concentrations of NaCl solutions were applied twice a day for a period of 5 days. After 5 days, leaves of treated and control plants were harvested for further investigations. For studying the morphological, physiolocial and biochemical changes the leaves were collected from the base of the plants under stress and controlled conditions.

Morphological characterization

To ascertain the comparative behaviour of different genotypes under salinity stress, observations were recorded on five randomly selected plants from each replication for five quantitative parameters such as plant height (cm), Number of leaves, Length of leaves (cm), number of nodes and length of internodes (cm).

Physiological characterization

Physiolocial traits were selected on the basis of plant water relations, which include leaf relative water content (RWC), excised leaf water retention (ELWR), and initial water content (IWC). These measurements were collected from three plants per genotype from each replication for eacth treatment after the treatment period. RWC, IWC, ELWR were measured using 3mm leaf disks which were weighed (fresh weight) and then placed in distilled water for 4 hours and re-weighed to obtain turgor weight (TW). The turgid leaf pieces were oven dried at 60^{o}c, and the dry weight (DW) was obtained after 24 hours of oven drying. RWC was calculated using the formula proposed by Ritche et al. (1990): %RWC = (FW-DW) / (TW – DW) x 100. IWC was calculated by measuring the fresh weight of leaf disks and dry weight after 24 hours of oven drying at 60^{o}c using the formula IWC = (FW-DW)/FW x 100. For measuring ELWR, the leaf disks were weighed and then kept at 30^{o}c for five hours and reweighed to obtain wilted weight. ELWR was calculated using the formula, ELWR = [1-(FW-WW4h)/FW] x 100, wehre, WW4h is the wilted weight after 4 hours.

Biochemical characterization

Selected biochemical parameters were used as a measure of salt stress responses in *S. melongena* and *S. insanum* (L) Prain. Amount of chlorophyll, caroteniods, phenol, catalase (CAT), peroxidase (POD), polyphenol oxidase (PPO) and ascorbic acid oxidase were estimated from fresh leaf tissues harvested from the seedlings after the period of stress treatment.

Estimation of chlorophyll and carotenoids

Chlorophyll pigments were estimated spectrophotometrically using the method of Arnon (1949) and calculated as follows:

Amount of Chl_a, mg/g tissue = $12.7\ (A_{663}) - 2.69\ (A_{645})$ x V/1000 x W

Amount of Chl_b, mg/g tissue = $22.9\ (A_{645}) - 4.68\ (A_{663})$ x V/1000 x W

Amount of total chlorophyll, mg/g tissue = $20.2\ (A_{645}) + 8.02\ (A_{663})$ x V/1000 x W

Total caroteniod content was also determined in the same chloroplast pigment extracted by 80% acetone measuring the absorbance at 510 nm and 480 nm. The amount of total caroteniod was calculated according to the equation given by Lichtenthaler (1987) and expressed as milligram of caroteniod per gram of plant tissue according to the formula: Amountof caroteniod, mg/g tissue = $7.6\ (A_{480}) - 1.69\ (A_{510})$ x V/1000 x W

where, A – Absorbance of specific wavelength

V – Final volume of chlorophyll extract in 80% acetone

W – fresh weight of tissue extracted

Estimation of total phenol

The total phenolic contents of leaf extract were determined according to the method described

by Malik and Singh (1980). A standard calibration plot was generated at 650 nm using known concentration of catechol. The concentrations of phenol in the test samples were calculated from the calibration plot and expressed as mg catechol equivalent of phenol/g of sample.

Enzyme Assays

Assay of catalase (CAT)

Catalase enzyme activity was assayed following the method described by Luck (1963).

Reagents: 0.067M phosphate buffer (pH 7.0); Hydrogen Peroxide-Phosphate Buffer: 0.16ml ofH_2O_2 (10% w/v) was diluted to 100ml with phosphate buffer and the absorbance of the freshly prepared solution was 0.5 at 240nm with a 1 cm light path. Enzyme Extract: 0.5g leaf tissue was homogenized in a blender with 5ml of phosphate buffer (assay buffer diluted 10 times) at 4°C. The sediment was stirred with cold phosphate buffer; allowed to stand in the cold with occasional shaking and centrifuged. The supernatants were used for the assay.

Procedure: The enzyme activity was measured spectrophotometrically at 240nm. The assay mixture contained 3ml of H_2O_2-phosphate buffer and 0.05ml of enzyme extract. Before use all the ingredients were maintained at room temperature against a control cuvette containing enzyme solution as in the experimental cuvette, but containing H_2O_2 free phosphate buffer. The time (Δt) required for a 0.05 unit decrease in absorbance due to enzyme activity was noted and the activity was expressed as units per mg protein per min. The protein content was estimated by the method of Lowry *et. al.* (1951).

Assay of Polyphenol oxidase (PPO)

Polyphenol oxidase enzyme activity was measured spectrophotometrically following the method of Taneja and Sachar (1974).

Reagents: 0.1 M phosphate buffer (pH 7.0); 0.1 M catechol; enzyme extract: 1g of fresh leaf tissue was homogenized in 2ml of 0.1 M potassium phosphate buffer (pH 7.0) containing 1% polyvinyl pyrrolione and centrifuged at 15000xg for 5 minutes at 4°C.

Procedure: The enzyme activity was measured spectrophotometrically at 430nm. The incubation mixture contained 2ml of 0.1 M potassium phosphate buffer (pH 7.0); 0.5ml of 0.1 M catechol and 0.5ml of enzyme extract. Before use all the ingredients were maintained at 37°C for 5 minutes. Omission of the substrate from the assay mixture served as control. The increased absorbance, due to enzyme activity was noted and the activity was expressed as units per mg protein per min. The protein content was estimated by the method of Lowry *et. al.* (1951).

Assay of peroxidase (POD)

Peroxidase enzyme activity was measured by Guaiacol method as described by Putter (1974).

Reagents: phosphate Buffer 0.1 M (PH 7.0); guaiacol Solution 20mM; hydrogen peroxide solution (0.042% = 12.3mM) freshly prepared; with the extinction of 0.485 at 240nm. Enzyme Extract: 1g of fresh leaf tissue was extracted in 3ml of 0.1M phosphate buffer (pH 7.0) by grinding with a pre-cooled mortar and pestle. The homogenate was centrifuged at 18000 g at 4°C for 15minutes and stored on ice till the assay was carried out.

Procedure: 1g of plant leaf tissue stored at -20°C, was extracted with 1ml of phosphate buffer (pH 7.0) by grinding in a pre-cooled glass mortar and pestle at 0-4°C. The homogenate was centrifuged at 18000g at 4°C for 15 minutes. The supernatant was used as enzyme source immediately. 3ml buffer solution (pH 7.0), 0.05ml guaiacol solution and 0.1ml enzyme extract and 0.03ml H_2O_2 solution were taken in a cuvette. Temperature of the mixture was raised to 25°C. After mixing the reaction mixture, the cuvette was placed in Spectrophotometer at 436nm. Time required in minutes (Δt) for increase in the absorbance of the assay mixture by 0.1 was read with the help of a stopwatch. Enzyme activity per ml of extract was calculated as follows: Enzyme activity (units/ml) = (3.18 x 0.1) / (6.39 x 1 x Δt x 0.1), where 6.39-extinction coefficient of guiacol. Activity, units per ml were then converted to per mg protein by estimating the protein content of extract by Lowry's method (Lowry *et. al.*, 1951). The estimated one unit of peroxidase was equivalent to that quantity of enzyme content per ml of leaf extract, which changes the OD of the reaction mixture by 0.1 in 60 seconds.

Assay of Ascorbic acid oxidase

Ascorbic acid oxidase was measured spectrophotometrically following Oberbacher and Vines (1963).

Reagents: 0.l M phosphate buffers (pH 5.6 and 6.5 separately); ascorbic acid solution: 8.8mg ascorbic acid in 300ml phosphate buffer (PH 5.6); enzyme extract: 0.5g of leaf tissue was homogenized in 2.5ml of 0.l M phosphate buffer (pH 6.5) and the homogenate was centrifuged at 15000 rpm for 15minutes. The supernatant was used as enzyme source. All the procedures were carried out at 4°C.

Procedure**:** 3ml of ascorbic acid solution was pipette out in a cuvette and read as reference in spectrophotometer at 265nm. 0.1 ml of enzyme extract was added to the reference cuvette. The decrease in absorbance at 265nm was read in 30 seconds intervals for 5 minutes. From the linear phase of reaction, the change in absorbance per min was calculated. Ascorbic acid had an absoption co-efficient of 760 $M^{-1}cm^{-1}$ at 265nm; so, the concentration of the ascorbic acid in the sample = change in OD per minute/ 760. The enzyme activity was expressed as µmoles of ascorbic acid oxidized (unit)/g tissue/minute.

Statistical Analysis

For each set of experiment, three replicants were maintained and the data were expressed as Mean ± standard eroor (SE). Student's t-test was used to estimate the significant differences at $P<0.01$ and $P<0.05$, between control and stress treatments.

Results

Effect of salt stress on plant growth and development

S. melongena and *S. melongena* var. *insanum* showed differential salt tolerance in terms of plant growth and development parameters. After two weeks of salt treatment, statistically significant reduction of plant height was observed in *Solanum melongena.* The difference was significant at 5% level in T1, while it was significant at 1% level in T2 and T3 treatments as against to that of control. The number of leaves showed significant reduction in T3 treatment (at 1% level), while leaf length did not show any significant reduction when compared to that of control. Significant reduction in the length of internodes were observed in T2 (at 5% level) and in T3 (at 1% level) as against to that of control (Table 1).

S. melongena var. *insanum* showed more tolerance towards salt tolerance in terms of plant growth and development parameters when compared to that of *S. melongena*. Salt treatment did not induce any statistically significant reduction in plant height as against to that of control (Table 2). However, the T3 treatment significantly reduced the number of leaves when compared to that of control (at 5% level). Reduction in the length of internodes were also observed in T2 and T3 treatments as against to that to that of control.

Effect of salt stress on plant physiology

Physiolocial characterization of salt stress responses in *S. melongena* and *S. melongena* var. *insanum* has been done in terms of IWC, RWC and ELWR. All the parameters recorded highest values in *S. melongena* var. *insanum* irrespective of the concentration of salt. Physiolocial parameters also indicated a high level of salt tolerace for the wild variety due to its high relative water content when compared to that of cultivated *S. melongena*. However statistically significant differences (at 1% level) were observed between the control and treated samples irrespective of the salt concentrations, in both genotypes (Table 3). The relative water content in the control plants of *S. melongena* var. *insanum* was 83.3% which became significantly reduced to 77.7% in T3 treatment while the control plants of *S. melongena* recorded a relative water content of 78.26% which became significantly reduced to 75% in T3 treatment.

Effect of salt stress on plant biochemistry

Photosynthetic pigments

Estimation of photosynthetic pigments showed a significant reduction with increase in salt concentration in salt treated plants of both genotypes when compared to that of controls. However *S. melongena* var. *insanum* showed higher amount of chlorophyll and caroteniod pigments in both control and treated plants, when compared with *S. melongena*. Total chlorophyll content in the control plants of *S. melongena* var. *insanum* was 0.185 mg/g tissue which showed a significant reduction to 0.174 mg/g tissue in T3 treatment. Similarly in *S. melongena* the total chlorophyll content was 0.175 mg/g leaf tissue in the control plants, which reduced significantly to 0.162 mg/f leaf tissue in T3 treatment (Table 4). A highly significant (at 1% level) reduction was observed in the amount of total caroteniods in the salt treated plants of both genotypes.

Total Phenolics

Estimation of total phenolics showed statistically highly significant increase with increase in salt concentration when compared to that of control in both genotypes. *S. melongena* var. *insanum* possessed high amount of phenolics in the control as well as treated samples when compared to that of *S. melongena*. In *S. melongena* var. *insanum*, the total phenolics in the control plants was 40.27 mg catechol equivalents / tissue, which significantly increased to 56 mg catechol equivalents / g tissue in T2 treatment. In *S. melongena* the amount of total phenolics was 35.55 mg catechol equivalents / g tissue in the control plants, which gradually increased to 36.27, 44.82 and 54.91 mg catechol equivalents/g, tissue in T1, T2 and T3 treatments respectively.

Enzymatic activities

S. melongena var. *insanum* showed significant increase in the catalase activity in treated plants

when compared to that of control. In T1, the increase in activity was statistically significant at 5% level, while in T2 and T3, increase in the activity was significant at 1% level. However *S. melongena* showed a significant (at 5% level) increase only in T3 treatment (Table 5). Peroxidase activity showed statistically significant increase in *S. melongena* with increase in salt concentration. However in *S. melongena* var. *insanum* peroxidase activity significantly increased in T2 and T3. In *S. melongena* ascorbic acid oxidase showed significant increase in treated plants irrespective of salt concentration when compared to that of control. While in *S. melongena* var. *insanum* significant increase was found only in the T2 and T3 treatments. Assay of polyphenol oxidase did not show any significant differences between the control and treated samples in both genotypes.

Table 1
Morphological characterization of *S. melongena* before and after the application of salinity stress

Parameters	Before treatment	After one week of treatment				After two weeks of treatment			
		Control	**T1**	**T2**	**T3**	**Control**	**T1**	**T2**	**T3**
Plant Height (cm)	10.2 ± 0.11	10.5 ± 0.06	10.5 ± 0.08	10.4 ± 0.06	10.4 ± 0.05	11.2 ± 0.06	11.0 ± 0.06*	10.9 ± 0.06**	10.8 ± 0.09**
Number of leaves	6 ± 0.40	7 ± 0.40	7 ± 0.40	6 ± 0.28	6 ± 0.28	9 ± 0.40	8 ± 0.28	8± 0.40	7 ± 0.028**
Length of leaves (cm)	9.5 ± 0.08	9.7 ± 0.08	9.6 ± 0.04	9.6 ± 0.04	9.5 ± 0.40	9.9± 0.07	9.8 ± 0.13	9.8 ± 0.05	9.7 ± 0.06
Number of nodes	5 ± 0.40	6 ± 0.40	6 ± 0.28	5 ± 0.28	5 ± 0.28	8 ± 0.40	7 ± 0.28	7 ± 0.28	75 ± 0.28
Length of inter nodes (cm)	2.6 ± 0.14	2.8 ± 0.07	2.7± 0.028	2.6 ± 0.06	2.6 ± 0.56	3.0 ± 0.09	2.8 ± 0.10	2.7 ± 0.02*	2.6 ± 0.06**

*Significant at 5% level
**Significant at 1% level

Table 2
Morphological characterization of *S. melongena* var. *insanum* before and after the application of salinity stress

Parameters	Before treatment	After one week of treatment				After two weeks of treatment			
		Control	**T1**	**T2**	**T3**	**Control**	**T1**	**T2**	**T3**
Plant Height (cm)	8.3±0.06	8.6 ± 0.08	8.6 ± 0.02	8.5 ± 0.02	8.5 ± 0.08	9.4 ± 0.06	9.4 ± 0.10	9.2 ± 0.10	9.2 ± 0.08
Number of leaves	5±0.28	6 ± 0.40	6 ± 0.28	5 ± 0.40	5 ± 0.40	8 ± 0.28	7 ± 0.40	7 ± 0.28	6 ± 0.40**
Length of leaves (cm)	8.6±0.101	8.8 ± 0.04	8.8 ± 0.04	8.7 ± 0.11	8.6± 0.06	9.7 ± 0.09	9.6 ± 0.14	9.6 ± 0.10	9.5 ± 0.08
Number of nodes	4±0.17	5 ± 0.28	5 ± 0.40	4± 0.40	4± 0.28	7 ± 0.28	6± 0.40	6 ± 0.40	6 ± 0.40
Length of inter nodes (cm)	2.3±0.074	2.4 ± 0.04	2.3± 0.08	2.3 ± 0.09	2.3 ± 0.01	2.8 ± 0.07	2.6 ± 0.02	2.5 ± 0.05*	2.4 ± 0.12*

Significant at 5% level (P<0.05)
**Significant at 1% level (P<0.01)

Table 3
Physiological characterization of *S. melongena* and *S. melongena* var. *insanum* under salinity stress

Parameters	Treatments	*S. melongena*	*S. insanum*
IWC	**Control**	84.21	86.36
	T1	83.33**	85.71**
	T2	82.35**	85**
	T3	80**	83.33**
RWC	**Control**	78.26	83.3
	T1	77.27**	81*
	T2	76.19**	80**
	T3	75**	77.7**
ELWR	**Control**	93.83	94.35
	T1	93.41	94.00
	T2	92.83	93.60
	T3	92.45	93.15

*Significant at 5% level (P<0.05)
**Significant at 1% level (P<0.01)

Table 4
Amount of photosynthetic pigments in *S. melongena* and *S. melongena* var. *insanum* under salinity stress

Parameters	Treatments	*S. melongena*	*S. insanum*
Chl_a (mg/g leaf tissue)	**Control**	0.168	0.171
	T1	0.165	0.169
	T2	0.161*	0.166
	T3	0.159*	0.164*
Chl_b (mg/g leaf tissue)	**Control**	0.006	0.010
	T1	0.005	0..008
	T2	0.003	0.007
	T3	0.002	0.005
Total Chl (mg/g leaf tissue)	**Control**	0.175	0.185
	T1	0.170	0.181
	T2	0.163*	0.177*
	T3	0.162*	0.174*

Carotenoids (mg/g leaf tissue)	**Control**	0.357	0.411
	T1	0.342*	0.393*
	T2	0.285**	0.388**
	T3	0.267**	0.347**

*Significant at 5% level (P<0.05)
**Significant at 1% level (P<0.01)

Table 5
Enzymatic activity and total phenolics in *S. melongena* and *S. melongena* var. *insanum* under salinity stress

Parameters	Treatments	*S. melongena*	*S. insanum*
Peroxidase activity	**Control**	0.074	0.083
	T1	0.103*	0.099
	T2	0.105**	0.103*
	T3	0.106**	0.108**
Catalase activity	**Control**	0.213	0.272
	T1	0.189	0.301*
	T2	0.225	0.336**
	T3	0.242*	0.331**
Polyphenol activity	**Control**	0.227	0.287
	T1	0.182	0.291
	T2	0.261	0.325
	T3	0.296	0.319
Ascorbic acid oxidase activity	**Control**	0.116	0.655
	T1	0.179*	0.681
	T2	0.237**	0.751*
	T3	0.278**	0.793**
Total Phenolics (mg catechol equivalents/g)	**Control**	35.545	40.27
	T1	36.27*	47.18**
	T2	44.82**	56.18**
	T3	54.91**	56**

*Significant at 5% level (P<0.05)
**Significant at 1% level (P<0.01)

Discussion

On a world scale, no toxic substance restricts plant growth more than does salt (Zhu, 2007). The present study focused on the physio-morphological and biochemical analysis of leaf extracts of cultivated and wild varieties of brinjal, *Solanum melongena* and *Solanum melongena var.insanum* under salt stress. Salinity treatment decreased morphological characters such as height of plant, number of leaves, length of leaves, number of nodes and length of internodes in the present study. Sairam and Tyagi (2004) suggested that salt stress negatively affects most plant growth phases and alters development. Plants examined in the present study, showed necrosis of mature leaves whose intensity increased with the concentration of the salt. Chlorosis starting from the leaf tips and moving up to the left stalk later become necrosis. Qadus (2011) reported that the treatment of sodium chloride reduced the number of leaves when compared with control plants in bean plant (*Vicia faba* L.). These results have been confirmed in earlier studies also in *Cicer arietinum*, Cowpea and wild bean (Karen et al., 2002; Raul *et. al*., 2003). Salinity affects plant growth and development because of low uptake and accumulation of essential nutrients and high accumulation of toxic ions such as Na^+ and Cl^- (Sabir and Ashraf, 2008). If excessive amount of salt enter the plant in the transpiring leaves, this may cause further reduction in growth (Greenway and Munns, 1980). Krasanky and Jonak (2012) suggested that the susceptibility of plants to salinity is also because of changes in molecular programs that affect development. The high concentration of ions such as Na^+,Cl^-, $Mg2^+$,SO_4^{2-} in saline soil inhibit the growth and development of many plants.

Initial water status is the main factor affecting the plant growth and development. Fricke *et al.* (2006) reported that within minutes following salt shock, water deficit and wilting occur because of rapid change in the osmotic potential difference between the plant and exterior environment. Present study also showed a gradual reduction in the amount of RWC, IWC and ELWR. Bastias *et al.* (2004) have reported that high concentration of salt disrupt homeostasis in water relations and change the ion distribution at both cellular and whole plant levels.

Relative water content is the direct measure of the water content of the plant, and it is considered as a reliable criteria to measure water in plant tissues, because relative leaf water and transportation rate have direct relation with the cell volume (Schonfeld *et al*., 1988). Present study has shown gradual reduction in percentage of RWC. Similar results were shown by Shaheen *et al.*, (2013) in *Solanum melongena* when comparing the RWC of wild and cultivated brinjal, where the wild brinjal showed high RWC. Siddique *et. al.* (2000) reported that the cause of higher RWC in tolerant plants as the ability to absorb more water from the soil and compensate transpiration done from plant leaves. It is thought that plants with high RWC have a more stable osmotic balance (Morgan, 1984). Present study showed a gradual reduction in percentage of ELWR value with increase in salt concentration. It may be inferred that that creation of drought stress conditions enabled the genotype for greater solute accumulation and osmotic adjustment (Lilley and Ludlow, 1996).

The present study showed that the total chlorophyll, chlorophyll a, chlorophyll b and carotenoid content gradually decrease with high concentration of salt. Similar results were obtained by Singh *et al.* (2016) in *S. melongena*. Reduction in chlorophyll content during salt stress occurs due to transportation of sugars and carbohydrates towards immature parts (Aggrwal *et al*., 2013). Decrease in total chlorophyll content may be observed due to ion accumulation and functional disorders observed during stoma opening and closing under salinity stress (Seeman and Critchley, 1985). Another reason for the decrease of chlorophyll content under salt stress as stated to be the rapid maturing of leaves (Yeo *et. al.*, 1991).

Phenolic compounds categorized as secondary metabolites essential for growth and reproduction of plants. They are known as hydrophilic antioxidants, and are produced as a response for defending injury of plants against pathogens. In the present study also, the total phenolic contents gradually increased with increase in salinity. Similar results were obtained by Lim *et al.* (2012) on buck wheat (*Fagopyrum esculentum* M.) sprout.

It is well established that like many other stresses, salinity stress also causes oxidative stress in most plants. Salt toxicity influences complex biochemical responses and several defensive mechanisms including production of enzymatic as well as non enzymatic antioxidants, which detoxify ROS that rapidly occurs in plants due to increasing salt concentration. Increased activities of many of the antioxidant enzymes in plants combat oxidative stress induced by salinity stress (Sharma *et al.*, 2012).

Peroxidases are enzymes able to catalyze reduction of hydrogen peroxide and oxidize various substrates. In the present study, the

enzyme peroxidase, polyphenol oxidase and catalase gradually increased with increase in salt concentration. When comparing both wild and cultivated brinjal, these enzymes are present at highest level in wild brinjal, *S. insanum*. The reduction in reactive oxygen species formation and increase of antioxidant defense system has been related to important mechanism for salt tolerance. Chawla *et al*. (2013) reported that oxidative stress defenses occur through enzymatic antioxidant mechanism including catalase (CAT), superoxide dismutase (SOD), peroxidase (POX) and enzymes of the ascorbate- glutathione cycle and non enzymatic antioxidants as phenolics and flavonoids. Of this some are scavengers of superoxide (O^{2-}) to form H_2O_2 and O_2, and plays an important role in defense activity against the cellular damage caused by environmental stress (Meloni *et. al*., 2003). Peroxidase involve in many physiological processes like responses to biotic and abiotic stresses, biosynthesis of lignin etc. Kalir *et al.* (1984) reported that the increased peroxidase activity might be an useful adaptation for prevention of peroxidation of membrane lipid by free radicals.

Catalase is a common enzyme found in nearly all living organisms. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). Present study showed catalase activity increased with increasing salinity. Similar results were also observed in Trigonella (Pour *et al*., 2013). In pistachio under varying salt treatment, the activities of CAT and other enzymes increased with increasing salt stress, indicating a protective mechanism against oxidative damage by maintaining an inherited and induced activity of antioxidant enzymes (Abbaspour, 2012). Polyphenol oxidase is copper protein of wide occurance in nature. In the present study no significant difference was obtained in the activity of PPO between control and treated plants of both genotypes.

In the present study, *S. melongena* var. *insanum* showed superior salt tolerance when compared to that of *S. melongena* in terms of plant growth development parameters as well as physiological and biochemical parameters evaluated. Similarly, drought and salt tolerances in wild relatives of wheat and barley have been reported earlier (Munns and Richards, 2007; Colmer *et. al*., 2006; Farooq, 2009). Rich genetic diversity provides the basis of evolutionary change by natural selection under environmental changes and stresses. Early domestication of crops created a bottleneck for gene diversity. While selecting important traits for cultivation, the process inadvertently lost others that might be useful in biotic as well abiotic stress resistance. Hence, development of crops with abiotic stress tolerance is needed to stabilize the production level globally. Present results suggest the use of wild relative *S. melongena* var. *insanum* as a reservoir of new alleles to confer stress tolerance to cultivated species of *S. melongena*.

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