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Effect of salinity on biochemical components of the egg plant (*Solanum melongena*)

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ABSTRACT

Present study explored the effect of salinity stress on seedling mineral composition, chlorogenic acid and polyphenol oxidase content of the two genotypes (GT25 and GT26) of egg plant (*Solanum melongena* L.). Selected egg plant genotypes were exposed to salinity stress (25-150 mM NaCl) along with control. Plants were grown in the field of School of Biotechnology, Gautam Buddha University during 2018–19. Drastic impacts of salinity stress as preliminary symptom were seen on seed germination. Genotype GT25 and GT26 were proficient to germinate only up to 100 mM and 75 mM NaCl respectively under salinity treatment. Results showed very poor growth along with necrotic/ dead tissue in the germinated seedlings leaves after the 30 days salt treatment in GT26 in100 mM NaCl treatment. Accumulation of Na+ ions is comparatively lower (67%) in GT25 under 75 mM NaCl concentration. On the contrary mineral (Cu, Mn, K) content, enzymatic activity like chlorogenic acid and polyphenol were resulted higher in GT25 compared to GT26 when subjected to NaCl stress (75 mM). These results indicate that egg plant genotypes respond to salt induced oxidative stress by enzymatic defense systems. The accumulation of polyphenol and chlorogenic acid suggest a role in protectivemetabolites. Hence it can be concluded that the GT25 possess strong tolerance against salt stress and could be an important genotype resource for the salt tolerance breeding programme of egg plant.

Keywords: Chlorogenic acid, Egg plant, Mineral content, Polyphenol oxidase, Salinity stress

Agricultural productivity worldwide is subjected to increasing environmental constraints, particularly to salinity due to its high magnitude of impact and wide distribution. Salinity inhibits crop growth through complex traits that include osmotic stress, mineral deficits and physiological and biochemical defects. In addition, excessive uptake of Na and Cl may result in a limited assimilation of mineral nutrients within the plants (Munns 2008, Puyang 2015). Phenolic compounds are secondary metabolites with high antioxidant capacity and play an important role in the protection of plants under abiotic stress against oxidative injury (Ahmad *et al*. 2010). Chlorogenic acids (CGA) which have antioxidant capacity observed by Wang *et al*. (2009), Zhang *et al*. (2008) and Sun *et al*. (2015). In plant, environmental stresses (biotic and abiotic) as salinity lead to accumulation of other phenolic constituents also such as polyphenol. Polyphenol oxidase (PPO) is an enzyme which oxidizes some phenols to chinone. In presence of atmospheric oxygen and PPO, monophenol is hydroxylated to o-diphenol and diphenol can be oxidized to o-quinones which then undergo polymerization to yield dark brown polymers. During adverse environment induced by high salinity stress these two enzymes CGA, PPO might be involved in the prevention of oxidative damage in plant and therefore could be an essential index for the adaptive mechanism in adverse circumstances.

Egg plant (*Solanum melongena* L.) is moderately sensitive to salinity (Heuer *et al*. 1986) but more attention to salinity is required in agricultural production with egg plant and its varieties. If the salt tolerance of egg plant varieties is known, it may be possible to minimise salt injury during the more sensitive, germination and seedling stages. In this paper, we present the effect of NaCl on minerals and biochemical component PPO, CGA in egg plant two genotypes, viz. GT25 and GT26 with an aim to access their tolerance ability and mechanism against salt stress. Salt treatment shows more effects in sensitive GT26 than salt tolerant GT25. Hence, GT 25 genotype could be utilized as potent salt tolerant genetic stock in future research and breeding programme.

MATERIALS AND METHODS

Thirty egg plant genotypes were procured from the Germplasm Exchange and Policy Unit, ICAR-National

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Bureau of Plant Genetic Resources (NBPGR), Pusa Campus, New Delhi and screened to test the best vigour on the basis of germination and early seedling growth stage. To investigate the effect of salinity stress on egg plant, two genotypes (GT25 and GT26, with accession number IC354140 and IC354562 respectively) were selected. Seeds of selected genotypes (GT25 and GT26) were surface sterilized with 0.1% $HgCl_2$ solution. In the seedling experiments, seeds of genotypes GT25 and GT26 were sown in a seedling pot size: 35 cm × 35 cm × 15 cm filled with standard soil: farm yard manure (2:1) mixture. The experimental design was completely randomized block with three replications. To impose stress in the genotypes, salinity levels of 0, 25, 50, 75, 100, 125 and 150 mM NaCl were used. The seedlings were watered with tap water up to the three-four leaf stages (30 days), and then were watered every 2 or 3 days for the next 30 days with solutions (50 ml per pot) of 0, 25, 50, 75, 100, 125 and 150 mM NaCl, prepared in distilled water (125 and 150 mM NaCl application showed stress effects on the growth of seedlings). Seedlings were harvested and washed with distilled water and measured the biochemical characteristics.

Biochemical parameters determination: Estimation of Copper, Manganese: The 60 days old seedling leave samples were dried in oven at 65±5°C and ground thoroughly. A representative ground plant sample (0.5 g) was taken for digestion. The samples were soaked overnight with 10 ml of concentrated HNO3 in conical flasks (100 ml capacity) for pre-digestion and finally digested in a di-acid mixture (20 ml) containing HNO3 and HClO4 acid (9:4). Kept the acid digested mixture on hot plate in acid proof digestion chamber at 100°C for 1 h and then at 200°C to continue digestion till the appearance of white colorless fumes. Remove the flasks from hot plate, cool and add 30 ml DDW and filtered through whatman no.42 filter paper. The volume was made up to 25 ml and stored in a container (100 ml capacity) for further analysis.

Estimation of Potassium and Sodium: The K and Na content in the standard solutions and plant samples were estimated by using K and Na - specific filters in a flame photometer (Systronics 128). By plotting a standard curve with known concentration of K and Na, the content of K and Na were calculated in seedling.

Estimation of Chlorogenic Acid (CGA): Standard solution preparation: For the standard solutions preparation a commercially bought CGA (catechol) were dissolved in polar-solvents (ethanol/methanol/acetonitrile and water). Prepared CGA (catechol) standard as concentration 0.1, 0.2, 0.3, 0.4 in µg/ml of distilled water. 500 mg of treated sample (seedling of GT25 and GT26 genotypes) were homogenized in 2 ml of chilled water. Homogenate was centrifuge at 8,000 rpm for 5 min at 4ºC, and collected the supernatant. Supernatant was washed two times with DCM (Dichloro methane) and kept for store at -20ºC in dark place. Double distilled water (DDW) used as blank as well as measure the absorbance at 324 nm and calculated CGA concentration. Estimation of CGA was followed the method as suggested by Belay and Gholap (2009).

Determination of Polyphenol oxidase enzyme assay (PPO): Enzyme extraction: The seedling of both genotype (GT25 and GT26) with different salt treatment were collected, washed thoroughly and blotted**.** The 100 mg sample was homogenized in 50 mM sodium phosphate buffer (*p*H 7.0). The filtrate then centrifuged at 12,000 rpm for 10 min and stored at -20°C. In order to determine the PPO activity, the modified method was used as suggested by Fujita *et al*. (1995). The reaction was initiated by the addition of 0.1 ml of the enzyme extract. The spectral absorption at 425 nm was recorded every min using a spectrophotometer for 5 min. Activity was determined by the increase in absorbance at 425 nm due to guaiacol oxidation. The following equation was used to calculate oxidase activity: $\Delta Abs/ (\varepsilon \times d) (10^3) (VT/VS)$ = Units/ml; Where: ΔAbs= change in absorbance per minute (for first 5 second), Ɛ= molar absorbance of the product (3450/M/cm), d= path length of light through sample, (VT/VS) = Volume of reaction mixture/Volume of enzyme, 10^3= conversion from mol/L to µmol/ml, U/ml= mol/min/ml. Results of the experiments were expressed based on three independent determinations. All results are presented as the mean values ± standard errors. Differences among treatments were considered significant at $P \leq 0.05$ after statistical analysis.

RESULTS AND DISCUSSION

Effect of salt stress on seedling biochemical parameters: The effects of salinity stress on the biochemical characteristics of the salt treated (NaCl) eggplant seedlings were evaluated. 30 days old seedlings of both genotypes (GT25 and GT26) were treated with salt treatments (25-150 mM NaCl) along with control (0) and observation was recorded after 30^{th} days of salt treatment. We observed that as the salt concentration increases (25–15 0mM NaCl), germination percentages were decline in both the genotypes. Drastic impacts of salinity stress as preliminary effect were

Table 1 Germination Percentage (GP) of egg plant (*Solanum melongena* L.) genotypes GT25 and GT26 under different salt treatment

Genotype	Germination percentage (%)						
	Salt concentration (NaCl mM)						
	0 (control)	25 mM	50 mM	75 mM	100 mM	125 mM	150 mM
GT25	90%	85%	70%	55%	40%	5-8% Poor-germination	No germination
GT26	90%	70%	45%	35%	2-5% Poor germination	No germination	No germination

Table 2 Effect of salt treatments (25 mM-150mM NaCl) on fresh weight and dry weight of 60 days old seedlings of egg plant genotypes (GT25 and GT26)

Genotype (GT)	NaCl (mM)	Seedling fresh wt. (mg)	Seedling dry wt. (mg)
GT25	0	11.4±0.007	5.21±0.007
	25	11.32±0.010	5.11±0.014
	50	11.21±0.007	5.06±0.008
	75	10.98±0.014	5.02±0.010
	100	10.80±0.014	4.99±0.010
	125	0.898 ± 0.013	0.009 ± 0.010
	150	0.078 ± 0.011	0.002 ± 0.013
GT26	0	6.40±0.021	3.87±0.004
	25	6.04±0.017	3.81±0.007
	50	5.93±0.029	3.61±0.010
	75	5.64±0.031	3.52±0.010
	100	0.222± 0.019	0.012±0.009
	125	0.019± 0.009	0.006 ±0.002
	150		

seen in terms of seed germination. Under salinity treatment genotype GT25 and GT26 were capable to germinate only up to 100 mM and 75 mM NaCl respectively. Poor germination (5–8%) was recorded in GT25 in 125 mM NaCl treatment, whereas GT26 seeds were failed to germinate in the same salt concentration (Table 1). Results of GT26 showed poor growth along with necrotic/dead tissue on the 60 days old seedling in 100-125 mM NaCl concentrations, whereas no seedling survived in 150 mM treatment. Seedling fresh and dry weight decreased with increasing salt concentration as compared to control in both the genotypes after the 30 days salt treatment.Among them, genotype 25 (GT25) had soaring biomass compared to GT26 (Table 2).

Mineral concentrations in both the genotypes of eggplants were measured. The sodium (Na^+) content is 67% less in GT25 than GT26 under salt condition (75 mM), on the contrary mineral (Cu, Mn, K) content was less in GT26 by 73%, 71%, and 59% respectively than GT25. GT26 show highly stressful effects on the seedling in 100 mM NaCl application, whereas GT25 showed comparative better growth in same concentration, indicating that both genotypes varied in the magnitude of their responses to salt. Seedling from both the genotypes subjected to different levels of salt stress was assayed for chlorogenic acid content (Fig 1A). CGA accumulation increased gradually with increasing concentrations of NaCl in both genotypes. The largest increase in CGA content compared to control plants was observed in seedling leaves of both genotypes treated with 75 mM NaCl ($P<0.05$). The increase in CGA content in the GT25 and GT26 was 8- and 3-time folds respectively as compared to control, which showed that GT26 was more sensitive to salinity than the GT25. Significant effects of salt eggplant seedling of both genotypes were found for the polyphenol oxidase enzyme assay (Fig 1B). Compared with the control, the activities of enzyme increased with salinity treatment. On the basis of obtained results we concluded that GT26 genotype showed low performance in each parameter can considered as salt sensitive genotype (SSG), while GT25 genotypes showed high value of all the studies parameters can considered as salt tolerant genotype (STG) of eggplant. The enzymatic activity of chlorogenic acid and polyphenol were 69.23% and 83.67% less in GT26 as compare to GT25 when subjected to high level of NaCl stress (75 mM). The results appeared to indicate that an adaptive response by the species of plant was created and the higher synthesis of CGA, PPO was observed to serve as the factor in adverse environmental situation.

The main aim of this study was to recognize the salt tolerance potential of selected genotypes at the early stage (seedling) of plant growth. In our experiments micronutrient concentrations were significantly affected (Cu, Mn decreased in seedling). These results were earlier observed by Saied *et al*. (2005). The solubility of micronutrients such as Cu and Mn can be reduced by NaCl salinity, but their uptake can be accelerated under the salt stress. Frequently, plants exposed to NaCl inevitably absorb a large amount of Na, which subsequently causes a decrease in the contents of K.

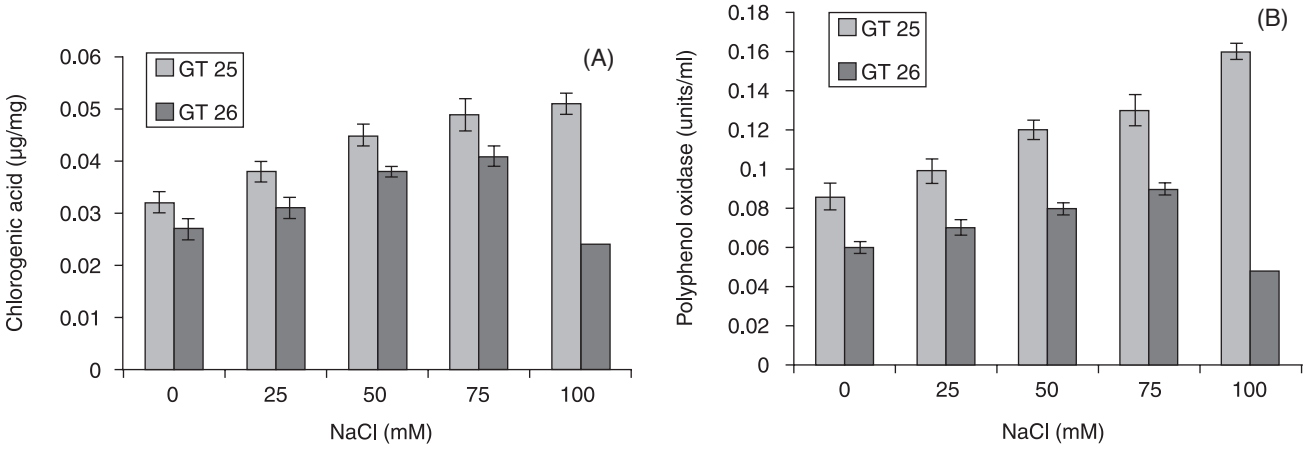

Fig 1 A. Effect of salinity level on Chlorogenic acid (µg/mg) in 60 days old seedling of eggplant genotype GT25 and GT26. B. Effect of salinity level on polyphenol oxidase (units/ml) in 60 days old seedling of eggplant genotype GT25 and GT26.

When the salt concentration increased, Na+ concentration in the seedling increased and K content decreased (Rabie and Almadini 2005). In agreement with the results by Zhao *et al*. (2015) CGA level was increased with the increase of salt stress (Fig 1A). In field experiment as salinity increased from 25 mM to 100 mM, the content of CGA reached to a maximum amount. Polyphenol oxidase enzymes are increased with salinity stress. Increased polyphenol oxidase activity under stress indicates the ability to oxidize and degrade the toxic substance such as phenolic compounds which are generally accumulated during salt stress. Increase in phenolic compounds response to salinity has also been reported in extract of different tissues of some other plants. Therefore, significant increase in the accumulation of chlorogenic acid and PPO activity in response to salinity suggesting that these phenolic compounds are perhaps stress-induced in eggplant. This could help to reduce oxidative pressure, this observation supports the hypothesis that, due to their polyhydroxyl nature, chlorogenic acid and PPO contributes significantly to the antioxidant activity of the eggplant. Same conclusion was also reported by Sgherri *et al*. (2007). We found that the GT25 had a higher CGA, PPO concentration than GT26. Antioxidant activity was found to be an effective determinant of salt tolerance in the set of eggplant genotypes examined. Therefore, enzymatic antioxidant defense system can protect plant cells from injury. These effects could be related to the tolerance mechanisms of eggplant and its varieties that were the subject of this experiment.

The present study concludes that salt stress induced changes in mineral (Cu, Mn, K and Na), CGA, PPO content. Salt treatment sharply decreased mineral (Cu, Mn and K) content in GT26-genotypes than GT25-genotypes. Both the genotypes (GT25 and GT26) showed an increased in Na, CGA, PPO activities under salt condition. However, these increases were higher in Accession no. IC354140 (GT25-genotype) than IC354562 (GT26-genotype). Hence on the basis of low and best performance of both the genotypes under high salinity levels, we concluded that the genotype GT25 is more tolerant to salinity stress in comparison with the genotype GT26. Suggestively, the CGA, PPO accumulation activity may be used as tolerance parameters for seedling screening while determining the salt tolerance of eggplant genotypes.

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