Seedling growth and antioxidant activity of eggplant (*Solanum melongena* L.) genotypes under salt stress

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Abstract

Salinity induces the abiotic stresses which affects plant cell metabolism and reduces plant productivity. This research was carried out in order to investigate the effect of salt stress on seedling growth and antioxidant activity among two genotypes of eggplant (Solanum melongena L.). Salt stress treatments were applied using salt solutions with 0 (control), 25, 50, 75, 100, 125 and 150 mM NaCl. In experiment, the effect of different concentration measured of salt level on germination percentage, seedling length, seedling fresh and dry weight. Results of the study showed that salt stress induced changes in antioxidant enzymes, superoxide dismutase (SOD) and peroxidase (POD), chlorophyll content and proline concentration. Salt treatment sharply decreased seedling length, fresh weight, dry weight and chlorophyll content in GT26-genotype when compared with GT25genotype. Both the genotypes (GT25 and GT26) showed an increased in proline, SOD and POD activities under salt condition. However, these increases were higher in GT25 than GT26. These results indicate that eggplant genotypes respond to salt induced oxidative stress by enzymatic defence systems.

Keywords: Salt stress, genotypes, eggplant, physiological parameters, proline, anti-oxidative enzymes

Introduction

Salinity induces abiotic stresses that negatively affect plant growth and development around the world particularly in arid and semi-arid regions. Salt stress had affected to decrease the germination percentage and germination rate in this crop, influencing an osmotic potential external to the seed preventing water absorption, or the toxic effects of Na⁺ and Cl[°] ions on germinating

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seed. Salinity inhibits seed germination and seedling establishment, crop growth and development, through complex traits that include osmotic stress, ion toxicity, mineral deficits, and physiological and biochemical defects, (Lin 2017). In addition, under saline conditions, osmotic and ionic stress leads to the production of reactive oxygen species (ROS) in chloroplasts, mitochondria, and the apoplastic space. The biosynthesis of the antioxidant enzymes (SOD, POD, PPO, MDA, CAT etc.) in plants was induced as defensive system, when exposed the salts. Superoxide dismutase (SOD) is one of the key enzymes responsible to diminish the concentrations of ROS and protect the cells against the oxidative damages (Hessini 2015). The control of the steady-state O_2^{-} levels by SOD is an important protective mechanism against cellular oxidative damage, since O₂⁻ acts as a precursor of more cytotoxic or highly reactive oxygen derivatives, such as per-oxy-nitrite or HO⁻. Therefore, SOD is usually considered the first line of defence against oxidative stress. Increased SOD activity had been correlated with increased protection from damage associated with oxidative stress. To understand the responses of eggplant under salinity stress and to reduce harmful effects on eggplant, seedlings were grown in salt stress condition. This research was conducted to investigate the changes in antioxidant activity of eggplant genotypes in salt stress condition.

Materials and Methods

Thirty eggplant (*Solanum melongena* L.) genotypes were procured from the Germplasm Exchange & policy Unit, ICAR - National Bureau of Plant Genetic Resources (NBPGR), Pusa Campus, New Delhi, and to test the best vigour on the basis of germination and early seedling growth stage (Table 1). To investigate the effect of salinity stress on eggplant, two genotypes (GT25 and GT26, with accession number IC354140 and IC 354562 respectively) were selected, based on their germination percentage. Seeds of selected genotypes (GT25 and

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GT26), were sterilized with 0.1% HgCl2 solution for 5 minutes, and then thoroughly washed two to three times with distilled water. In the seedling experiments, seeds of genotypes GT25 and GT26 were sown in a seedling pot size: 35x35x15 cm filled with standard soil: farm yard manure (2:1) mixture. with mean air temperature of $30/25 \pm 3^{\circ}C$ (day/night). The experimental design was completely randomized block with three replications. To impose stress in the genotypes of eggplant, salinity levels of 0 (tap water/ control), 25, 50, 75, 100, 125 and 150 mM NaCl were used. The seedlings were watered with tap water up to the threefour 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. Seedlings were harvested and washed with distilled water on the 60th day (125 and 150 mM NaCl application showed stress effects on the growth of seedlings) and measured the growth and physiological characteristics.

Physiological growth parameters: At the end of the experiment, seedlings were harvested and immediately assessed the fresh weight (FW). Subsequently, seedling length and root length were measured. After that, plant samples were dried at 70 °C for 48 h to constant weight to obtain plant biomass (dry weight, DW) per individual plant.

Determination of chlorophyll: For leaf biochemical measurements, collected the second fully expanded fresh leaf from the top during the growth stage after the 30 day of salinity treatment. Leaves samples were kept in ice bouquet to minimize the loss of moisture. All the observations were mean of three replications. Leaf chlorophylls (Chl) were extracted in 80% acetone and absorbance at 663 and 645 nm were measured. The chlorophyll a, chlorophyll b, and total chlorophyll quantities were calculated according to the method of **Arnon (1949)**, using the formula as follows:

Chl. a (mg g⁻¹ fwt.) = $[12.7 (A663) - 2.69 (A645)] \times V/$ 1000 × W; Chl. b (mg g⁻¹ fwt.) = $[22.9 (A645) - 4.68 (A663)] \times V/$ 1000 × W; total Chl. (mg g⁻¹ fwt.) = $[20.2 (A645) + 8.02 (A663)] \times V/$ 1000 x W (Where, A = optical density (nm); V = final volume of chlorophyll; W = fresh weight).

Determination of free proline: Proline content in leaf tissues was measured via reaction with ninhydrin (Bates *et al.* 1973). For colorimetric determinations, a solution of proline, ninhydrin acid and glacial acetic acid (1:1:1) was incubated at 100°C for 1h. Then, the reaction was cooled in an iced bath. The chromophore was extracted using 4ml of toluene and its absorbance at

520nm was spectrophotometrically determined with toluene as the blank.

Measurements of antioxidant enzyme activities: For antioxidant enzyme extractions, 0.2 g of fresh leaves was homogenized with 0.1M phosphate extraction buffer. The filtered homogenate was then centrifuged at 15,000 g for 20 minutes at 4 °C, and the resulting supernatant was used to evaluate the activity of superoxide dismutase (SOD, EC 1.15.1.1), and peroxidase (POD, EC 1.11.1.7). Both the enzyme activities were measured by an UV/Visible Spectrophotometer (Thermo Scientific 220). SOD activity was assayed by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) using the method of Dhindsa et al. (1981). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of NBT reduction. The POD activity was determined as described by Castillo et al. (1984) using guaiacol as a substrate. One unit of POD activity was defined as the amount of enzyme that increased the absorbance at 470 nm by 0.01 absorbance unit per minute. For statistical analysis the experimental design was completely randomized. All results are presented as the mean values \pm standard errors. For each extract, the absorbance was determined on duplicate assays.

Results and Discussion

The results of germination percentage are presented in Table 1. In our study we observed that the highest seed germination (90-100 %) was observed in two genotypes [IC354140 (GT25); IC 354562 (GT26)], while the lowest percentage of seed germination (0-30 %) noted in 21 types of genotypes. On the basis of our results we classified the selected 30 genotypes into four groups, as high (IC354140-GT25, IC 354562-GT26), moderate (IC345747-GT22, IC350885-GT23); susceptible (IC111415-GT16, IC261814-GT20, IC354135-GT24, IC354707-GT28, IC383372-GT30) and highly susceptible genotypes (Table 2). Genotypes GT25 and GT26 showed the greatest germination percentage therefore, to study the effect of salinity stress on eggplant, we have selected these two genotypes of eggplant for further study. Germinated seedlings (30 days old) of both genotypes were treated with different salt treatments (25-150mM NaCl) along with control (0) and observation was recorded after 30th days of salt treatment. Germination percentage in the selected genotypes was drastically reduced under salt stress. We observed drastic impact of salinity in GT26 (germination 35% in 75mM NaCl) compared to GT25 (55%) (Table 3). We observed necrotic sites and dead tissue in the

seedling leaves (100mM NaCl) after the 30 days salt treatment in GT26. Seedling length, seedling fresh and dry weight and leaf area generally decreased with increasing salt levels (75 mM) as compared to control in both the genotypes (Table 4). Among them, genotype-25 (GT25) had the largest leaf area, root length and seedling biomass compared to GT26. Chlorophyll concentrations in both the genotypes of eggplants were measured. Chlorophyll a, chlorophyll b, and total chlorophyll concentrations decreased with increasing NaCl concentrations from control to 75mM NaCl (Fig 1) in GT26. GT26 showed highly stressful effects on the seedling in 100mM NaCl application, whereas GT25 showed comparative better growth in same concentration, indicating that both genotypes varied in the magnitude of their responses to salt. Leaves from both the genotypes, subjected to different level of salt stress were assayed for accumulated proline content. The proline contents of both genotypes treated with 0-150 mM NaCl are shown in Fig. 2 In general, proline accumulation increased gradually with increasing concentrations of NaCl in both genotypes. The largest increase in proline content compared to control plants was observed in plants of both varieties treated with 75 mM NaCl (P < 0.05). The increase in proline content in the GT25 was 8-fold compared to controls; it was 3 fold compared to controls in the GT26. When we compared these results between the selected eggplant genotypes, it was observed that the GT26 was more sensitive to salinity than was the GT25. Significant effects of salt eggplant leaves of both genotypes were found for the antioxidant enzyme activities (SOD and POD) (Fig. 3). Compared with the control, the activities of each antioxidant enzyme increased with salt level. On the basis of our obtained results we concluded that GT26 genotype showed low performance in each parameter can considered as salt sensitive genotype (SSG), while GT25 genotype showed high value of all the studies parameters can considered as salt tolerant genotype (STG) of eggplant.

The main aim of this study was to recognize the salt tolerance potential of selected genotypes at the early stage (germination and seedling) of plant growth. As was mentioned previously, the effects of salinity on plant growth can vary depending on the plant species and also on different genotypes of a species. Thus, for improving salt tolerance it is important to monitor the genetic variability of plant species among genotypes. We included early stages of plant development, because this is well documented that germination and seedling features are the most viable principles, and the final plant performance depends heavily on seedling specifications (Bybordi 2009). Germination percentage was significantly decreased by salt stress in eggplant genotypes. Many researchers have reported similar results (Datta et al. 2009). This also agreed with the result of (Gholamin and Khayatnezhad 2010) in wheat and Mostafavi (2011) in safflower. The decrease in germination rate particularly under drought and salt stress conditions may be due to the fact that seeds seemingly develop an osmotically enforced "dormancy" under stress conditions. This may be an adaptive strategy of seeds to prevent germination under stressful environment thus ensuring proper establishment of the seedlings. Salinity caused a significant reduction on seedling length. This is in agreement with previous reports in Wheat Akbarimoghaddam et al. (2011), Sorghum bicolor El Naim et al. (2012). Te reduction in root and shoot development may be due to toxic effects of the higher level of NaCl concentration as well as unbalanced nutrient uptake by the seedlings. Decrease of growth in root and shoot can be related to NaCl toxicity and disproportion in nutrient absorption by seedlings. Salt stress caused a decrease in the fresh weight and dry weight of shoot and root. This reduction was relatively dependent on shoot or root lengths. Yildirim et al. (2011) demonstrated that salt stress significantly decreased the plant fresh and dry weight of Physalis species. Datta et al. (2009) showed that different level of salinity significantly affected the growth





S. No	Genotype Accession Number	Germination (%)	Rating	Germination and growth	Accession Number-	Total no.
1	EC038474	0	-	incidence (%)	Genotype	
2	EC169079	0	1	High (90-100 %)	IC354140-GT25	2
3	EC305048	20			IC 354562-GT26	
4	EC379244	10	2	Moderately (60-89 %)	IC345747-GT22	2
5	EC384970	0			IC350885-GT23	
6	EC393239	10	3	Susceptible (30-59 %)	IC111415-GT16	5
7	IC089818	0			IC261814-GT20	
8	IC089890	0			IC354135-GT24	
9	IC089923	10			IC354707-GT28	
10	IC090144	10			IC383372-GT30	
11	IC090160	10	4	Highly Susceptible	EC038474-GT1	21
12	IC090785	10		(0-30 %)	EC169079-GT-2	
13	IC090905	0			EC305048-GT3	
14	IC111013	10			EC379244-GT4	
15	IC111033	10			EC384970-GT5	
16	IC111415	30			EC393239-GT6	
17	IC111439	10			IC089818-GT7	
18	IC112741	20			IC089890-GT8	
19	IC144145	20			IC089923-G19	
20	IC261814	40			IC090144-GT10	
21	IC279555	10			IC090160-GT11	
22	IC345747	70			IC090785-GT12	
23	IC350885	60			IC090905-GT13	
24	IC354135	40			IC111013-GT14	
25	IC354140	90			IC111033-GT15	
26	IC 354562	90			IC111439-G117	
27	IC354672	0			IC112/41-G118	
28	IC354707	30			IC144145-G119	
29	IC374852	20			IC2/9555-G121	
30	IC383372	40			IC3546/2-G12/	
	10303572	19			IC374852-GT29	

 Table 1: Germination Percentage (GP) of all genotypes

Table 2: Rating scale of 30 eggplant genotypes

Table 3: Germination Percentage (GP) of genotypes 25 and 26 under different salt treatment

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

Table 4: Effect of salt treatments (25 mM-150mM NaCl) on 60 days old seedling length, fresh weight and dry weight in two genotypes (GT25 and GT26) of eggplant (*Solanum melongena* L.)

Genotype (GT)	NaCl (mM)	Seedling length	Root length	Seedling fresh wt.	Seedling dry wt.
		(cm)	(cm)	(mg)	(mg)
	0	12.2 ± 0.04	6.2±0.07	11.4±0.007	5.21±0.007
	25	11.8±0.20	6.1±0.07	11.32±0.010	5.11±0.014
	50	11.6±0.20	6.03±0.04	11.21±0.007	5.06±0.008
GT25	75	10.9 ± 0.07	5.3±0.07	10.98±0.014	5.02±0.010
	100	10.6±0.07	5.1±0.07	10.80 ± 0.014	4.99±0.010
	125	-	-	-	-
	150	-	-	-	-
	0	11±0.10	9.53±0.04	6.40±0.021	3.87±0.004
	25	10.7 ± 0.04	9.1±0.07	$6.04{\pm}0.017$	3.81±0.007
	50	10.2±0.07	8.4±0.07	5.93±0.029	3.61±0.010
GT26	75	10 ± 0.07	8.03±0.04	5.64±0.031	3.52±0.010
	100	-	-	-	-
	125	-	-	-	-
	150	-	-	-	-

attributes by reducing the biomass and length of root and shoot. In our study, we found that NaCl concentrations greater than 75 mM caused chlorosis for the eggplant genotypes. The level of chlorosis increased with increasing NaCl concentrations in GT26. Chlorophyll content is a basic way to evaluate the effects of environmental stress (Silva-Ortega et al. 2008). Photosynthesis is the main ROS-producing process in

chloroplasts, and ROS can cause photo inhibitory and photo-oxidative damage. Chlorosis is a common response to salinity, and it causes the inhibition of photosynthesis. Thus, pigment degradation is a rapid indicator of plant response. Jampeetong and Brix (2009) treated 2 genotypes of Triticum aestivum with 200 mM NaCl for 5 days; they determined that chlorophyll a and b and total chlorophyll contents decreased. Glycine betaine (GB) and proline are the most common osmolytes produced in plants under salt stress; therefore, in our study, we evaluated proline accumulation. Proline accumulation and stress tolerance correlation have been reported in different studies, and it has been observed that proline concentrations are higher in stress-tolerant plants than in stress-sensitive plants (Misra and Gupta 2005). We found that the GT25 had a higher proline concentration than GT26. Salt-tolerant genotype generally show higher activity of these antioxidant enzymes as compared to salt sensitive ones (Sreenivasasulu et al. 2000). This suggests that high antioxidant enzyme activity has a significant role in imparting salt tolerance in plants. In this background, the higher SOD and POD activities in the seedling of Genotype 25 under salt stress signify its high tolerance to salinity stress. These results are substantially in agreement with those of Sairam et al. (2005) who reported a lower decrease in membrane stability index in tolerant genotypes of wheat than in salt sensitive ones under salt stress. In our study, antioxidant activity was found to be an effective determinant of salt tolerance in the set of eggplant genotypes examined in the present study, because it showed a positive correlation between antioxidants activity and seedling dry weight or shoot and root lenght but negative correlation obtained with seedling dry weight (Table 4). If reactive oxygen species in plant were not removed in time, plants would be subjected to seriously oxidative damage. Therefore, enzymatic antioxidant defense system can protect plant cells from injury. SOD and POD are the most important protective enzymes to remove reactive oxygen species.

As shown Table 1-2 reflects that GT25, GT26 showed best germination percentage 90% under control (without salt treatment) as compared to the other selected genotypes but after salt treatment of these genotypes, GT26 did not cop with high salt concentrations (35% in 75mM NaCl) when compared with GT25. It was observed that necrotic sites and dead tissue in the salt treated seedling leaves of GT26. Germination percentage, seedling length, fresh and dry weight, leaf area was drastically decreased with increasing salt levels in GT26. Further proline accumulation and stress tolerance correlation is the one of the most important parameters to screen the salt tolerant / salt sensitive genotypes. Misra and Gupta 2005 observed that proline concentrations are higher in stress-tolerant plants than in stress-sensitive plants. Our results showed same resemblance and found that the GT25 had a higher proline concentration than GT26. Based on our results and literature details we can use salt sensitive genotype for GT26-genotype instead of poorly germinating genotype.

Conclusion

This study interprets that control treatment of the 30 accessions/ genotypes showed clear differences in germination percentage. Accession no. IC354140 (GT25-genotype) and IC 354562 (GT26-genotype) showed the highest seed germination, under salinity stress condition and adapted towards salt stress. High salt concentration (100mM) affects the germination of eggplant seeds, also retard the early phases growth of seedlings, induce changes in antioxidant enzymes (SOD and POD), chlorophyll content and proline concentration. Salt treatment sharply decreased seedling length, fresh weight, dry weight and chlorophyll content in GT26genotype than GT25-genotypes. Both the genotypes (GT25 and GT26) showed an increased in proline, SOD and POD activities under salt condition. Hence on the basis of low and best performance of both the genotypes under high salinity levels, we concluded that the GT25genotype is more tolerant to salinity stress in comparison with the GT26-genotype. Suggestively, the proline accumulation and anti-oxidative enzymes (SOD and POD) activity may be used as tolerance parameters for seedling screening while determining the salt tolerance of eggplant genotypes.

सारांश

मृदा लवणता, प्रमुख अजैविक प्रतिबलों में से एक है जो पादप कोशिका चयापचय को प्रभावित करता है और पादप उत्पादकता को कम करता है। यह शोध बैंगन (*सोलनम मेलॉन्गेना* एल.) दो प्रभेदों (जीनोटाइप्स) में नवोद्भिद अंकुर वृद्धि और एंटीऑक्सिडेंट गतिविधि के प्रतिबल प्रभाव की जाँच करने के लिए किया गया। नमक प्रतिबल प्रशोधन हेतु नमक के विभिन्न घोलों जैसे–0 (नियंत्रण), 25, 50, 75, 100, 125 और 150 मिमी सोडियम क्लोराइड का उपयोग किया गया। प्रयोग में हमनें नमक के स्तर की विभिन्न सांद्रता पर अंकुरण प्रतिशत, अंकुर की लंबाई, अंकुर के ताजा और सूखे वजन के अंकुरण पर प्रभाव को मापा गया और परिणाम से पता चला है कि नमक तनाव द्वारा एंटीऑक्सिडेंट एंजाइमों एस.ओ.डी. और पी.ओडी. क्लोरोफिल सामग्री और प्रोलीन एकाग्रता में परिवर्तन को प्रेरित किया है। नमक उपचार द्वारा नमक सहिष्णु जी.टी. 25 प्रभेद के मुकाबले नमक संवेदनशील जी.टी. 26 प्रभेद में अंकुर की लंबाई, ताजा वजन एवं सूखा वजन और क्लोरोफिल की मात्रा में कमी पायी गयी। दोनों प्रभेदों (जी.टी.25 और जी.टी. 26) ने नमक के दबाव के तहत प्रोलीन, एस.ओ.डी. और पी.ओ.डी गतिविधियों में वृद्धि दिखाई। हालाँकि यह वृद्धि जी.टी. 25 में जी.टी. 26 की तुलना में अधिक थी। इन परिणामों से संकेत मिलता है कि बैंगन के जीनोटाइप नमक प्रेरित ऑक्सीडेटिव प्रतिबल एंजाइम रक्षा प्रणालियों द्वारा प्रतिक्रिया दिखाते हैं। इसलिए यदि इस विशेषता के माध्यम से जीनोटाइप का चयन किया गया तो मृदा तनाव की स्थिति में उपज को बढ़ाया जा सकता है।

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