Effect of Glycine Betaine on Morphological and Physiological Attributes of Tomato (*Lycopersicon esculentum* L.) Cultivars under Saline Conditions

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Abstract. Tomato (*Lycopersicon esculentum* L.) is a long duration crop belongs to a family Solanaceae. In case of vegetables, tomato is a second major crop, cultivated wide range throughout the world. Although, tomato is moderate sensitive to salinity yet for salinity tolerance more attention is required. More than 30% cultivated land all over the world severely affected by the salinity. In this scenario, experiment was designed to investigate various morphological and physiological aspects of tomato under various salinity levels; different levels of exogenous glycine betaine applications. Study was conducted to reveal the salt tolerance in tomato genotypes. Experiment was performed under controlled condition in the growth chamber of the IHS, UAF. Different concentrations of sodium chloride salt (0, 1.5 and 3 dS m⁻¹) was used for salinity levels. Medium size plastic pots were used for sowing of tomato and sand was used as growing medium. Hoagland solution was applied for nourishment of tomato seedlings. Salinity was applied on 3-4 leaf stage. Then examined the effect of glycine betaine (0, 5, 10 and 15mM) for salt tolerance on tomato cultivars. Data of various attributes was collected and analyzed statistically by appropriate statistical package. Results revealed that tomato growth was negatively affected by the salinity. Morphological attributes and physiological attributes reduced in response to salinity except electrolyte leakage which amplified in salt stress. Exogenous application of glycine betaine promotes the tolerance against the salinity in the tomato genotypes and enhance growth.

Introduction

Tomato from family solanaceae is one of the chief vegetal crop, cultivated worldwide. Globally, tomato was cultivated on 4.8 million hectares with production of a 178 million tonnes in 2016 [1]. Tomato production during 2013-14 in Pakistan was 599 thousand tonnes [2]. This crop is cultivated in broader range of climatic conditions, but most favorable areas for cultivation are warm and relatively dry [3] and in these arid and semiarid regions high soil salinity and water stress are the two common environmental stresses [4]. Environmental stress that inhibit the growth of crops salinity one of them [5]. Changes occurred in plant cell structure due to saline conditions [6,7] and unswervingly affected the biochemical attributes, physiological and morphological attributes of plants [8] Saline conditions affected the growth development, protein production and respiration [9,10].

Cope the salinity affect, plants adapted various changes in morphological and physiological attributes [11,12] Synthesis and gathering of compatible solutes (Osmolytes/metabolites) is common strategy in plants to protect them from environmental stresses [13]. The gathering of osmolytes help to overcome the oxidative damage triggered by free radicals produced as result of high salt stress and also sustain damage of subcellular structure.

Glycine betaine (GB) is one of the osmolyte which is quaternary ammonium compound and in some plants its accumulation is communal retort to lessen the environmental stresses [14]. Glycine betaine improve tolerance in crops against environmental stresses including salt stress either applied exogenously or by transgenic approach [15,16,17]. Glycine betaine improved acceptance in plants in contradiction of salt strain through mechanism by protecting membrane veracity, reduce oxidation in membrane lipids and activation of enzymes [18]. Glycinebetaine is extremely rich in chloroplast

and most vital part in resistance to thylakoid membrane and upholding photosynthetic efficacy [19]. Though, several techniques have been experienced on plants. These well-suited solutes hold water within cells and protect cellular sector from injury produced by dehydration, throughout water deficiency. GB recover crop growth and seedling development under water deficit conditions [20]. It is also used to increase the deficiency tolerance of several crops such as wheat, rice and cotton [21]. GB applied exogenously can improve the activities of anti-oxidative enzyme and plant has minor oxidative stress [22]. When the quantity of GB is low, carbon dioxide accommodation rise. Glycinebetaine also complicated in the osmotic adjustment, in cotton plants [23]. Likewise, GB rises strain tolerance by stopping photoinhibition and declining erosion of lipid membranes [24]. Glycine betaine also helpful in ion homeostasis and Na^+/K^+ inequity when applied exogenously under salinity stress [25].

Hence, the current experiment was conducted to appraise the consequence of glycine betaine on two tomato genotypes under saline conditions by assessing their morphological and physiological attributes.

Material and Methods

This experiment was conducted to induce salt tolerance in two tomato genotypes (L00587 and L00602) by exogenously applied of glycine betaine at seedling stage. The research was carried out in Growth Chamber of Institute Horticulture Sciences of University of Agriculture Faisalabad, Pakistan. The seeds of two tomato genotypes were attained from Ayub Research Centre Faisalabad. There were four levels of GB (0, 5, 10 and 15mM) applied. The sowing was carried out in medium size plastic pots and as the growth medium sand was used; before sowing the growth medium was washed with adequate distilled H_2O to eradicate the salts already present in it. Hoagland's solution was applied as a source of nutrition. Through irrigation, salt stress was given after 20 days of germination of crop. Salinity level applied as 0, 1.5 and 3 dSm^{-1} respectively for stress and control conditions. After 7 days of salt application glycine betaine was applied. There were four levels of GB (0, 5, 10 and 15 mM). Completely randomized design with three repeats was used to conduct this experiment.

Following treatment were used:

- T₀= Control (Non-saline + No spray)
- T₁= 1.5 ds m^{-1} + No spray of glycine betaine
- T₂= 1.5 ds m^{-1} + 5mM of glycine betaine
- T₃= 1.5 ds m^{-1} + 10mM of glycine betaine
- T₄= 1.5 ds m^{-1} + 15mM of glycine betaine
- T₅= 3 ds m^{-1} + No spray of glycine betaine
- T₆= 3 ds m^{-1} + 5mM of glycine betaine
- T₇= 3 ds m^{-1} + 10mM of glycine betaine
- T₈= 3 ds m^{-1} + 15mM of glycine betaine

Subsequently 40 days of growth the seedlings were uprooted; morphological attributes (root and shoot length, number of leaves, plant fresh weight, plant dry weight) and physiological attributes (chlorophyll content, relative water content, osmotic potential, electrolyte leakage, water potential) were recorded. Recorded data were statistically scrutinized using ANOVA. Data collection methods mentioned in table 1.

Table 1. Morphological and Physiological attributes

Morphological Attributes	No. of plant leaves	After thirty five days of growth the seedlings were uprooted and washed with distilled water to remove the foreign particles of sand. Total number of leaves of seedling counted for each plant for each replication. At the end the average was computed.
	Measurement of root lengths (cm)	After forty days of growth the seedlings were uprooted and washed with distilled water to remove the foreign particles of sand. Root length of two randomly selected seedlings from each replicate were measured in centimeters (cm) from the base of hypocotyls to tip of the root with the help of meter rod. The average of each replication was calculated.
	Measurement of shoot lengths (cm)	After forty days of growth, the seedlings were uprooted and washed with distilled water to remove the foreign particles of sand. Shoot length of two randomly selected seedlings from each replicate were measured in centimeters (cm) from the base of hypocotyls to the tip of the shoot with the help of meter rod. The average of each replication was calculated.
	Plant fresh weight (g)	After forty days of growth, two plants from each replicate were gently up rooted and washed with distilled water to remove the sand particles. After washing these plants were wrapped with filter paper to remove any drop of water present on their leaves and shoots. Then these were placed on the digital balance for the calculation of fresh weights. And average fresh weight of each replicate was recorded.
	Plant dry weight (g)	After measuring the fresh weights the plant material from each replicate was taken in paper bags and then placed in dring oven (Memmert-110, Schawabach) dried at 72°C for a week of drying the dried biomass was calculated on digital balance and average dry weight of each replicate was taken.
Physiological Attributes	Chlorophyll contents	Chlorophyll a and b contents of the fresh material of plant were estimated from Aron method (1949). In eighty percent acetone 100 mg of the fresh material of plant was extracted. The measurements were made on spectrophotometer (Hitachi-U2001, Tokyo, Japan) using 645 nm and 663nm. The contents of chlorophyll a and b were calculated by using following formulae. $\text{Chl. a (mg g}^{-1} \text{ f.wt.)} = (1.27 (\text{OD}_{663}) - 2.699 (\text{D}_{645}) \times 5/1000 \times 0.1$ $\text{Chl.b (mg g}^{-1} \text{ f.wt.)} = (22.9 (\text{OD}_{645}) - 4.68(\text{OD}_{663}) \times 5/1000 \times 0.1$
	Water potential of leaf (-MPa)	After forty days of plant growth third fully expanded youngest leaf of ten plants (two from each replicate treatment ⁻¹) was cut with a razor at the petiole and placed in the gasket of in the morning before the 12.00 am (10.00 am to 12.00 a.m.).
	Osmotic potential of leaf (-MPa)	The same leaf that was used in pressure chamber for Osmotic potential was placed in a plastic bag and kept at low temperature (-20°C) in a freezer for a week. The frozen leaf material was then thawed at room temperature for half hour and cell sap was extracted with the help of a disposable syringe. The 10 µl of extracted sap was placed on osmometer (Wescor Model-5500) with the help of plastic syringe and Osmotic Potential measurement was taken.
	Relative water contents (RWC)	Fully prolonged leaves were sampled and weighed to take fresh leaf weight. To obtain fully turgid weight the leaf sample were placed into water for three hours. Then the leaves were removed from water and oven dried at 70% for 24 hours and noted the dry weight. Following formula was used to calculate RWC; $\text{RWC\%} = \frac{\text{fresh wt} - \text{dry wt}}{\text{turgid wt} - \text{dry wt}} \times 100$
	Electrolyte leakage (%age)	After forty days two plants from each replicate was taken and dipped in test tube. The distilled water present in the test tube and then measure the density of the plants from test tube. And then these test tube was autoclave at 121°C for 15 minutes in 15 psi. Then again measure the density of test tubes with Electro leakage meter.

Results

Morphological Attributes:

Number of leaves: Analysis of variance (ANOVA) for the data of number of leaves shows that salinity stress significantly reduces the number of leaves for both tomato genotypes. And with reference to this attribute both genotypes differ significantly. When exogenously glycine betaine applied both genotypes showed improvement and enhance number of leaves under saline conditions. (Fig. 1).

Root Length: Plant root length reduced significantly under the salt treatments but three i.e. 5mM, 10mM and 15mM level of glycine betaine improved this attribute of both genotypes. 15mM was most effective than 5mM and 10mM glycine betaine application (Fig. 2).

Shoot Length: Plant root length reduced significantly under the salt treatments but three i.e. 5mM, 10mM and 15mM level of glycine betaine improved this attribute of both genotypes. 15mM was most effective than 5mM and 10mM glycine betaine application (Fig. 3).

Plant Fresh Weight: Plant fresh weight of both tomato genotypes was expressively abridged by salinity conditions while the applications of glycine betaine induce the tolerance and improvement against salinity. 15mM application of GB more effective in L00587 as compare to L00602 (Fig. 4).

Plant Dry Weight: Plant fresh weight of both tomato genotypes was importantly abridged by salinity conditions while the applications of glycine betaine induce the tolerance and improvement against salinity. 15mM application of GB more effective in L00587 as compare to L00602 (Fig. 5).

Physiological Attributes:

Chlorophyll Content: In two tomato genotypes net considerable reduction of chlorophyll content observed under the salt treatment. And with reference to this attribute both genotypes differ significantly. Recovery showed as the result of exogenous application of glycine betaine (Fig. 6).

Water Potential: Statistical analysis for variance of the water potential attribute shows that salinity badly affected the water potential, but this attribute shows recovery as the result of the foliar application of glycine betaine. 5mM, 10mM 15mM glycine betaine exogenous application more effective under 1.5 ds m⁻¹ salt concentration (Fig. 7).

Osmotic Potential: Analysis of variance for osmotic potential indicates that this attribute showed the significant reduction against the salinity stress. As concerned this attribute, both genotypes showed significant difference. Application of glycine betaine improve the osmotic potential against salinity stress (Fig. 8).

Relative Water Content: Statistical analysis for variance of the relative water content attribute shows that salinity badly affected the relative water content percentage, but this attribute shows recovery as the result of the foliar application of glycine betaine. 15 mM glycine betaine more effective under 1.5 ds m⁻¹ salt concentration (Fig. 9).

Electrolyte Leakage: Electrolyte leakage jumped up under the saline condition; much higher in 3 ds m⁻¹ salinity level. This attribute reduced by the effect of glycine betaine under salinity stress. All levels of glycine betaine effective (Fig. 10).

Fig. 1. Effect of glycine betaine on number of leaves of tomato genotypes under salinity

Fig. 2. Effect of glycine betaine on plant root length of tomato genotypes under salinity

Fig. 3. Effect of glycine betaine on plant shoot length of tomato genotypes under salinity

Fig. 4. Effect of glycine betaine on plant fresh weight of tomato genotypes under salinity

Fig. 5. Effect of glycine betaine on plant dry weight of tomato genotypes under salinity

Fig. 8. Effect of glycine betaine on osmotic potential of tomato genotypes under salinity

Fig. 6. Effect of glycine betaine on chlorophyll content of tomato genotypes under salinity

Fig. 9. Effect of glycine betaine on relative water content of tomato genotypes under salinity

Fig. 7. Effect of glycine betaine on water potential of tomato genotypes under salinity

Fig. 10. Effect of glycine betaine on electrolyte leakage of tomato genotypes under salinity

Table 2. LSD mean comparison results of tomato genotypes for various traits

Treatments	NOL	PRL	PSL	PFWT	PDWT	CC	EL	RWC	WP	OP
T ₀	5.95 b	5.2348 cd	6.1292 b	0.8950 d	0.8567 b	9.107 d	73.173 d	77.057 d	1.2267 e	1.2088 c
T ₁	3.00 d	4.4100 f	4.5967 ef	0.7187 g	0.5200 c	5.563 h	59.873 g	68.257 h	0.8500 i	0.8133 g
T ₂	5.48 bc	4.7800 e	5.2650 cd	0.7817 ef	0.6150 c	8.007 f	64.870 f	73.913 f	0.9333 g	1.0300 e
T ₃	6.83 a	6.8167 b	8.2778 a	1.1208 b	0.9602 ab	10.677 b	81.090 b	81.433 b	1.4767 b	1.3667 ab
T ₄	7.00 a	7.1233 a	8.6233 a	1.3783 a	1.0683 a	12.613 a	83.910 a	85.010 a	1.5367 a	1.4000 a
T ₅	2.00 e	3.8867 g	4.1417 f	0.5983 h	0.4950 c	4.513 i	57.050 g	63.910 i	0.8233 j	0.7733 h
T ₆	5.20 c	4.6700 e	4.8600 de	0.7542 f	0.5517 c	6.687 g	60.050 g	71.833 g	0.8933 h	0.8700 f
T ₇	5.54 bc	5.1667 d	5.5917 c	0.8021 e	0.6583 c	8.660 e	68.167 e	76.093 e	1.0833 f	1.1433 d
T ₈	6.00 b	5.3783 c	8.2667 b	0.9936 c	0.9300 ab	9.947 c	75.097 c	78.007 c	1.3800 c	1.3400 b

NOL= No. of leaves, PRL= Plant root length, PSL= Plant shoot length, PFWT= Plant fresh weight, PDWT= Plant dry weight, CC= Chlorophyll contents, EL= Electrolyte leakage, RWC= Relative water content, WP= Water potential, OP= Osmotic potential

Discussions

Table 2. presented the mean comparison distribution among various traits under different treatments. Salt adversely affect the biomass of the two tomato genotypes and these results related to the previous studies that saline conditions during plant growth limited to the water absorption, reduce the biochemical reactions distributions and also caused decrease in leaf area [26]. So, these changes refereed to negative response of biomass of tomato plant. The adversative consequence on plant length, plant fresh and dry weight instigated by salinity in our experiment were observed. Results revealed that when glycine betaine applied exogenously in salinity conditions increase in plant fresh and dry weight occurred and the similar finding were found in many earlier experiments [10,27]. By the exogenous application of glycine betaine dry weight augmented because the photosynthetic action increased by glycine betaine. Zhang et al., [28] avowed similar findings. It has been observed by preceding studies that exogenous treatment of glycinebetaine enhanced the growth of plant under salinity and non-salinity conditions because of enhanced doings of antioxidant that lessen the stress hurt.

Number of leaves reduced is response to effect of salinity. Parvin et al., [29] and Jafari et al., [30] described similar outcomes. Glycine betaine help to reduce salinity and enhancing leaf numbers. Root and shoot length abridged in response to salt stress, alike these findings Kausar et al. [10] derived results and showed progress in response to exogenous application of glycine betaine.

This study indicated that relative water content diminished by the salinity conditions. Hu et al. [18] showed similar results and also Alasvandyari and Mahdavi [31] indicated these findings. The reduction in chlorophyll content recovered in response to glycine betaine effect [18]. Salinity deteriorated water potential and osmotic potential because Na^+/K^+ ratio disturbed by salt. Glycine betaine rehabilitate water potential and osmotic potential for proper cell functions [32].

The present study indicated that electrolyte leakage increased due to salt stress, because of more oxidative damage. Glycine betaine's exogenous application reduce this oxidative damage and help to reduce electrolyte leakage. Similar findings were reported in many earlier experiments [33]. Our results exhibited that exogenous application of glycinebetaine increased the salinity tolerance in tomato. In non-saline condition than in salinity conditions, external importance of glycinebetaine in fresh and dry weight of shoot and root recommended that glycinebetaine is strongly maintained in the plant growth parameter.

Conclusion

It can be determined based on results that salt stress putting adverse impact on the growth of tomato plant; its morphological and physiological attributes. Glycine betaine exogenously application significantly mitigating the deleterious properties of salinity condition. 15mM glycine betaine proved more effective as compare to 5mM and 10mM. As the end point glycine betaine induced resistance against salt stress in both tomato genotypes.

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