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EVALUATING PHYSIOLOGICAL RESPONSES OF TOMATO GERMPLASM TO TIME-INTERVAL SALT STRESS

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ABSTRACT: Effect of different salt stress on the physiological traits in eleven tomato (*Solanum lycopersicum* L.) germplasm was studied to assess the genotypic variation. Thirty days old laboratory grown seedling and seventy days old filed grown plants were supplemented with 0 mM, 20 mM, 50 mM, 75 mM, 100 mM, 125 mM and 150 mM salt concentration and incubated for 12 hrs, 24 hrs and 36 hrs. Electron leakage (EL), Na^+ accumulation and Guaicol Peroxidase (GPX) antioxidant activity were evaluated after 12 hrs, 24 hrs and 36 hrs at each salt stress conditions. Among studied traits, Na^+ accumulation was showed statistically significant variations and Na^+ accumulation was increased with increasing salt stress and stress duration period. No significant change in Na^+ accumulation was noted in TG7 at all salt stress conditions. EL and GPX parameter does not show any variation among the studied germplasm at all salt stress conditions at three time intervals. The results allow further understanding of the physiological parameters with the adaptation of the tomato germplasm to saline conditions.

KEYWORDS: Tomato, NBPGR, Time-interval salinity, Na^+ accumulation, Seedling stage.

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1. INTRODUCTION

Tomatoes (*Solanum lycopersicum*) are an economically important amongst the vegetable crops and used as fresh, cooked, pulp, paste, juice or like sauces [1]. However, tomato yield is seriously limited because of abiotic stress, such as salinity or drought [2]. Salt stress induces changes in various metabolic and physiological processes depending on duration of the stress and severity eventually inhibits crop production [3]. Salinity stress limits the productivity of agricultural crops, with

negative effect on germination, plant vigor and yield to crops [4]. All of these are associated with nutritional imbalance, oxidative stress or ionic/osmotic affects [5], [6], [7], [4]. According to [8], plants responded to salt stress are basically explained in three ways. These are causing water deficit by reducing water potential in the root zone, Na^+& Cl^- ions phytotoxicity and severe changes in shoot transport due to imbalance in uptake. Tomato is sensitive to moderate levels of salinity stress and salt affected areas were increasing rapidly [9]. Tolerance to abiotic stress (salinity) at the seedling stage is positively correlated with tolerance to abiotic stress in mature plants [10], [11], [12], [13] concluded that the yield reductions because of salinity may be due to both the 'osmotic stress' that initiates relatively high solute concentrations in the root growth, and 'specific toxicity' which maintains accumulation of high concentrations of Na and Cl in the plant, which of above causes physiological alterations and finally that inhibit plant biomass. Plants require molecular, physiological and biochemical strategies to survive this problem of salinity. It is an essential to understand how plants react to salt stress and adjust to this stress to avoid the yield loss. The aim of the study was to characterize the physiological response of 30 days-old seedlings as well as 70 days-old eleven tomato germplasm at different time-intervals salt stress. The detailed investigation of physiological parameters of plant against to short term salinity presented here will not only provide the information about response of plants to salt stress but also may be helps in selection of salt-tolerant and salt sensitive germplasm.

2. MATERIALS AND METHODS

Biological material and growth conditions:

The seeds of eleven tomato (*Solanum lycopersicum* L) germplasm named TG1 (NBPGR, EC 241446), TG2 (NBPGR, EC 251649), TG3 (NBPGR, EC 251581), TG4 (NBPGR, EC 315478), TG5 (NBPGR, EC 177297), TG6 (NBPGR, EC 398405), TG7 (NBPGR, EC 164329), TG8 (NBPGR, EC 523851), TG9 (NBPGR, EC 159053) TG10 (NBPGR, EC 631410) and TG11 (cv. Arka Vikas) were obtained from National Bureau of Plant Genetic Recourses (NBPGR), Rajendra nagar, Telangana, INDIA and Indian Institute of Horticulture Research (IIHR), Bangalore, INDIA. Uniform and healthy sized seeds of all varieties were surface sterilized with 4% sodium hypochlorite solution for five minutes, followed by ten repeated washings with distilled water. The sterilized seeds were sown in nursery for 21 days then after shifted to RBD (Randomized Blocking Model) model field and allowed to grown upto 70 days for Ontogeny stage experiments; seeds were placed in coconut peat contained egg shaped trays and allowed to grow upto 30 days for seedling stage experiments under laboratory conditions.

Physiological parameters measurements:

Traits like Guaicol Peroxidase (GPX) and Electron leakage (EL) parameters were estimated by followed the procedure of [14] and [15]. The accumulation of Na^+ content was measured with ELICO 378- Flame photometer. Above physiological traits were measured on the eleven tomato

germplasm at all salt stress condition along with control. These parameters were measured on three individual plants from three individual experiments and were used to calculate the mean values followed by statistical analyses

Stress application:

All seedlings were additionally supplemented with 0, 20, 50, 75, 100, 125 and 150 mM NaCl solution for different time intervals viz. 12 hrs, 24 hrs and 36 hrs. The fully expanded matured leaves of every plant were harvested, immediately stored at -80ºC until required for analysis.

Statistical analysis:

Each treatment was replicated thrice and triplicate results were used to construct graphs through Graph pad PRISM 5.0 software. The mean values for various parameters of the plants were subjected to statistical analysis following the standard procedure described by [16]. The means were compared in One-way ANOVA analysis by means of Tukey's multiple comparison tests for significant difference in order to study the significance at a $P \leq 0.01$ level of probability.

3. RESULTS AND DISCUSSION

Response of 30 days old tomato seedlings (Laboratory conditions) treated with different time interval salt stress:

The present study was carried out to understand the diverse response of tomato germplasm towards different salt stress conditions at 12 hrs, 24 hrs and 36 hrs time intervals. The eleven tomato germplasms were allowed to grow against short term salt treatments like 12, 24 and 36 hrs of time intervals and total three physiological parameters were evaluated. The difference between control conditions and salt treated conditions parameters within germplasm were evaluated for statistical significance ($P<0.01$) variations through one-way ANOVA analysis (Tukey's multiple comparison test).

Electron leakage and Guaicol peroxidase:

After 12 hrs of salt stress at control conditions, the TG4 and TG10 germplasms were having higher Electron Leakage like 67.94 ±1.71% and 62.16 ±0.87%, respectively. Whereas, TG1 germplasm had lowest electron leakage (40.7 ±1.08%) (Figure 1). With increasing the salt stress from 20 mM to 150 mM, the electron leakage was not significantly ($P<0.01$) varied in comparison to their controls after 12 hrs of salt stress. Even after 24 hrs and 36 hrs of different salt stress (20 mM to 150 mM) application does not induce significant ($P<0.01$) variations in the all studied tomato germplasm (Figure 1). In overall only 3-5% of increased electron leakage levels was observed after 36 hrs of stress but it is not statistically significant (Figure 1).The germplasms TG6, TG10 and TG9 were produced higher activity and TG3 and TG1 were showed lower GPX activity (Figure 1) in laboratory grown plants at control (non salinity) conditions. Guaicol peroxidase is one of the antioxidant enzymes which showed a gradual increased activity with increasing salt stress in all studied germplasm after 12 hrs of salt stress; however the increase is not statistically significant (Figure 1).

Antioxidant enzyme Guaicol peroxidase was appeared as an increased pattern in all studied germplasm at 24 hrs and 36 hrs short term salinity stress incubation at all salt concentrations (Figure 1). The Statistical one way ANOVA results are clearly explained that there no significant variations ($P<0.01$) in between control and any saline treatment in all germplasms. GPX have been reported as one of important antioxidant enzymes in different plant tissues [17]. As reported in many plants both enzymatic and non-enzymatic antioxidant plays an important role in scavenging the ROS. The results reveals that TG6, TG10 and TG9 were showed highest GPX activity at seedling stage. The findings of present research demonstrate that inherent activities of the antioxidant enzymes are occurred in all studied tomato germplasm leaves but are expressed differentially between germplasm. From this study we can say that enzymatic antioxidants (GPX) do play an important role in tomato. This variation of GPX was not stasticially significant at all salt conditions with respective controls (Figure 2). It has already been reported CAT and GPX plays a major role in ROS scavenging mechanism under salt stress as reported in Barley, Onion, French bean, Wheat, Rice and Horse gram [18], [19], [20], [21], [22], [23] and [24].

Na^+ accumulation:

Different salt stresses such as 20 mM, 50 mM, 75 mM, 100 mM, 125 mM and 150 mM were given to 30 days plants grown in laboratory condition and after12 hrs leaf samples were collected to estimated the Na^+ accumulation in all eleven tomato germplasm (Figure 2). At control condition the Na^+ accumulation was noted from 304.36 ±5.81 ppm (TG6) to 114.63 ±3.74 ppm (TG2) (Figure 2). All the germplasm when treated with 20 mM salt stress for 12 hrs doesn't show any significant increase in their Na^+ accumulation in leaves (Figure 2). However at 50 mM salt stress TG1, TG2, TG4, TG5, TG6, TG9, TG10 and TG11 germplasms showed significant ($P<0.01$) increase in Na^+ content and the germplasms TG3, TG7 and TG8 were not showed any significant increase ($P<0.01$) in comparison to their respective control plants (Figure 2). Twelve hrs of treatment of 75 mM, 100 mM, 125 mM and 150 mm salt stress enhance the Na^+ ion levels significantly ($P<0.01$) in all germplasm (Figure 2). The influx of Na^+ content were estimated after 24 hrs of salt treatment and observed a slender increased pattern of Na^+ accumulations in all germplasm due to various saline treatments (Figure 2). Except TG1 and TG11 remaining all germplasms, the Na^+ content was not increased significantly ($P<0.01$) in leaves at 20 mM. At 50 mM salt stress, except TG1, TG9 and TG11 remaining all germplasm were showed significantly ($P<0.01$) increased Na^+ content.

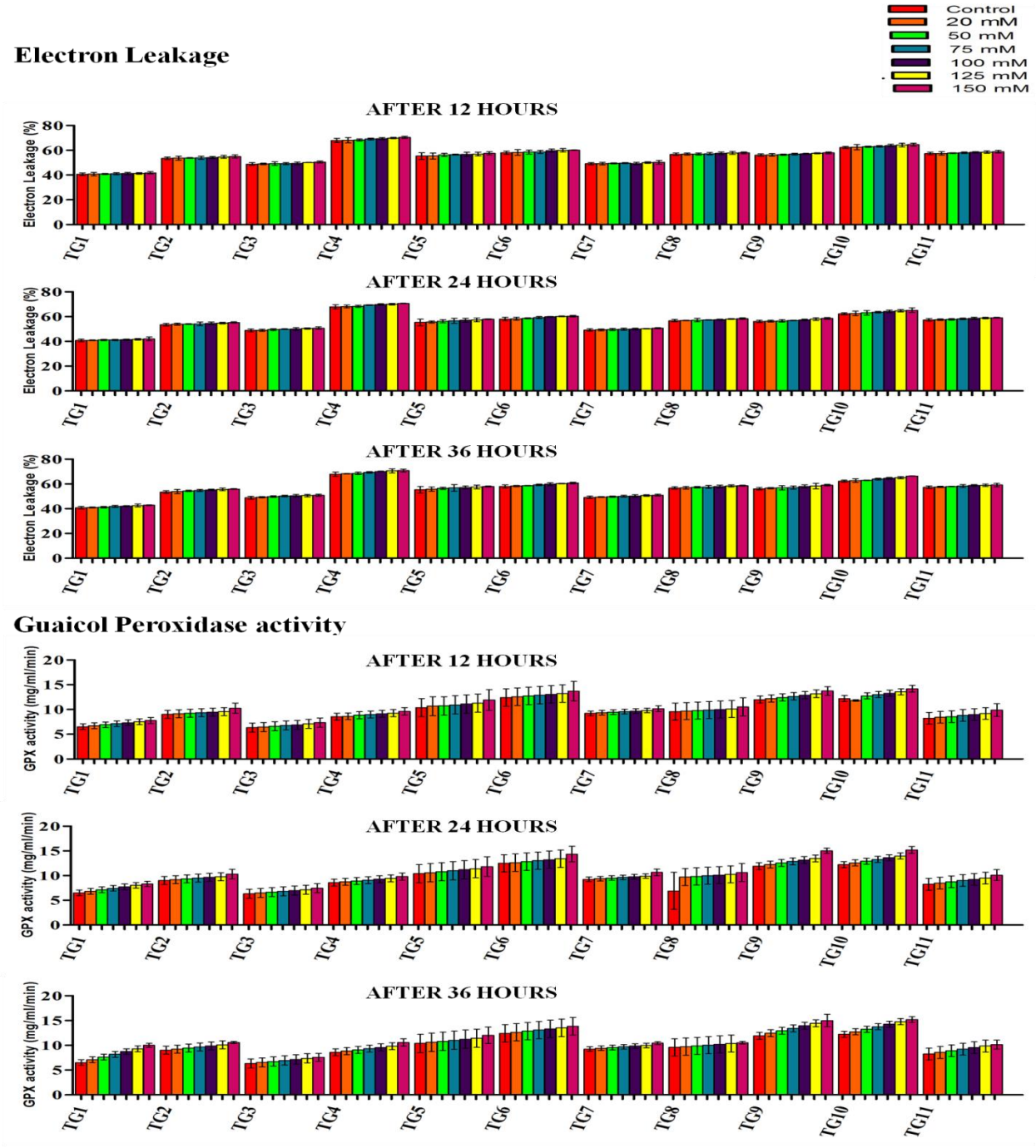

Figure 1: Effect of different time interval salinity (12, 24 & 36 hrs) on Electron Leakage and Guaicol Peroxidase activity in eleven tomato germplasm at 30 days old seedlings stage under laboratory conditions. Each bar represents the mean (± SE) of three measurements. Among all treatments statistically significant ($P \leq 0.01$) with their respective controls were presented with '*' on vertical bar.

TG5 along with TG2, TG3 and TG4 were not showed significant ($P<0.01$) increase in Na^+ content at 75 mM salt stress. The germplasm TG2, TG3 and TG4 didn't have any significant ($P<0.01$) variations in Na^+ content in leaves even at 100 mM salt stress. Only TG3 doesn't have significant ($P<0.01$) variation in Na^+ content still at 125 mM salt stress. When germplasm was evaluated after 24 hrs of salt stress, at 150 mM all the germplasm showed significant ($P<0.01$) increase in Na^+

content in leaves. Among all germplasm TG1 and TG11 were showed significantly (P<0.01) increased Na^+ content in at all studied salinity levels compared to respective controls (Figure 2). High influx of Na^+ content were found after 36 hrs of salt treatment and observed slender increased patterns of Na^+ accumulations in all saline treatments (Figure 2). When the germplasm screened at 20 mM salinity stress, except the germplasm TG1, TG9, TG10 and TG11 remaining all others were accumulated significant (P<0.01) increased Na^+ content in leaves (Figure 2). Only the germplasm TG7 didn't have significance (P<0.01) in Na^+ at 50 mM and 75 mM salt stress and remaining all germplasms were accumulated significant Na^+ content (Figure 2). All the germplasms were accumulating higher Na^+ content significantly (P<0.01) when screened at 100 mM, 125 mM and 150 mM (Figure 2). Moreover, to date, studies showing the important role of Na^+ exclusion in overall salt tolerance have been based mostly on shoot/leaf or even whole-plant Na^+ content [25], [26], [27], [28] and [29].

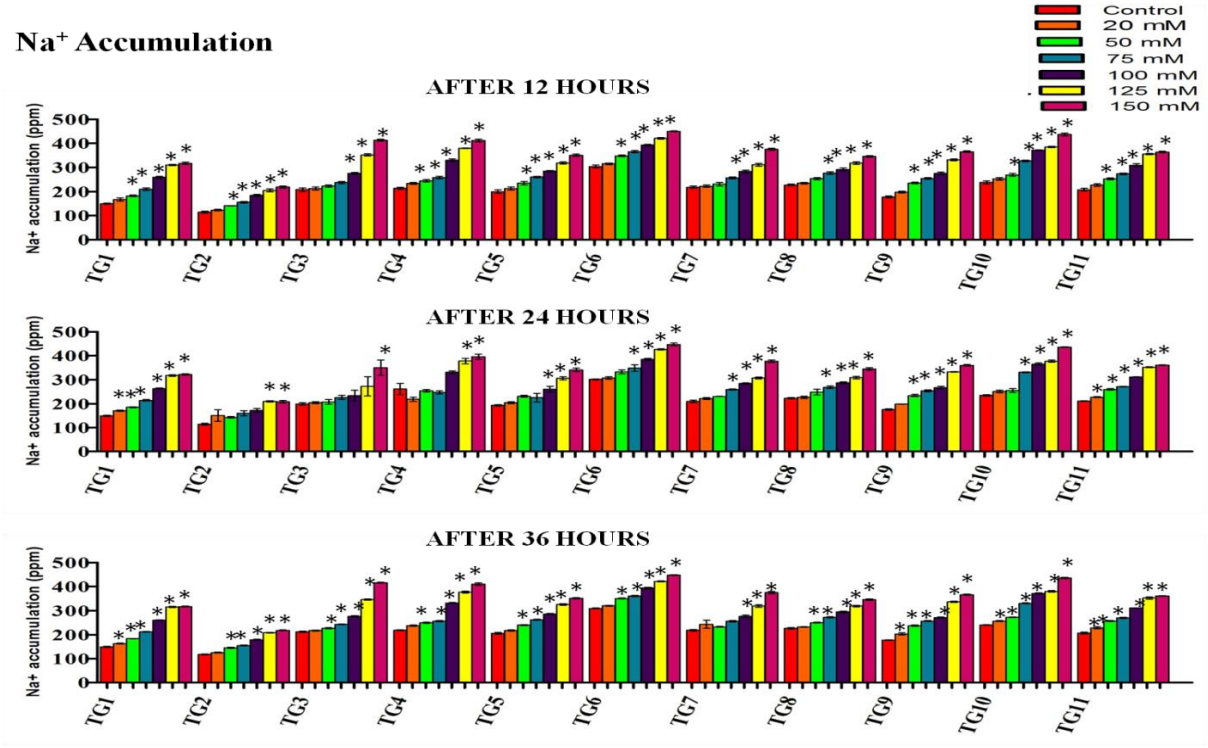

Figure 2: Effect of different time interval salinity (12, 24 & 36 hrs) on Na^+ accumulation in eleven tomato germplasm at 30 days old seedlings stage under laboratory conditions. Each bar represents the mean (± SE) of three measurements. Among all treatments statistically significant (P≤0.01) with their respective controls were presented with '*' on vertical bar.

Apart from shoot growth rate, the rate of recirculation of Na^+ to the roots via phloem has been suggested as an important factor affecting Na^+ concentrations in shoots. Whether this restricted Na^+ accumulation in shoot/leaves is achieved mainly by root Na^+ export or shoot Na^+ exclusion, or by both of these processes with tight regulation/coordination at different growth stages and time scales, however, has remained un-clarified. Under short term salt stress, TG7 & TG8 and TG3, TG4, TG5

TG6 and TG11 germplasms displayed the lowest Na^+ accumulation in leaves when compared to the TG1, TG9 & TG10 germplasms (Figure 3). The accumulation of ions requires the accumulation of solutes in the cytosol playing a role in both osmo-protection and osmotic adjustment under abiotic stress [30], [31] and [4].

Response of 70 days old tomato seedlings (Net house conditions) treated with different time interval salt stress:

Seventy days old tomato plants (Net house conditions) were treated with salt stress, to under how plants were response to different salt stress at early time period intervals. The tomato germplasms were allowed 12, 24 and 36 hrs of salt stress and three physiological parameters were evaluated. The difference between control conditions and salt treated conditions parameters within germplasm were evaluated for statistical significance ($P<0.01$) variations through one-way ANOVA analysis (Tukey's multiple comparison test).

Electron leakage and Guaicol peroxidase:

Among all studied germplasm highest electron leakage was found in TG6 (82.48 ±3.67%) & TG1 (80.62 ±3.71%) and followed by TG3, TG2 & TG9, whereas lowest was observed in TG4 (68.94 ±1.98%) & TG7 (70.64 ±1.1%) compared to other germplasm at non-salinity condition (Figure 3). The germplasm TG6 was showed highest EL at 20 mM (83.08 ±3.67%) and 50 mM (83.68 ±3.67%) salinity stress and followed by TG1 (Figure 3), However, after 12 hrs of salt stress incubation there is no significant ($P<0.01$) variation was noted in EL in all studied germplasm at all salinity conditions compared to respective controls. The Electron Leakage of the all studied germplasm was not significantly ($P<0.01$) affected at all salt stress conditions after 24 hrs of salt stress (Figure 3). Different salt stress (20 mM to 150 mM) does not induce significant ($P<0.01$) variations in Electron Leakage even after 36 hrs of stress in all the studied tomato germplasm (Figure 3).Guaicol peroxidase is one of the antioxidant enzymes which showed a gradual increased activity with increasing salt stress in all studied germplasm; however the increase is not statistically significant ($P<0.01$) (Figure 3). In control condition, TG8 (8.95 ±1.98 mg/ml/min) showed highest GPX activity followed by TG3 (7.81 ±1.87 mg/ml/min). GPX activity of eleven tomato germplasm were not significantly ($P<0.01$) changed in compared to control even at higher degree of salt stress after 12 hrs of salt stress. At all salt stress conditions TG8 was continued to produce highest GPX activity followed by TG3, whereas the germplasm TG5 and TG6 were showed low levels of GPX activity in all salt treatments (Figure 3). Even after 24 hrs of salinity incubation there is no significant ($P<0.01$) variation was observed in GPX activity in all germplasm at all salinity conditions (Figure 3). Antioxidant enzyme Guaicol peroxidase was appeared as an increased pattern in all studied germplasm after 36 hrs of short term salinity stress incubation (Figure 3) however the Statistical one way ANOVA results were clearly said that there is no significant variations ($P<0.01$) was observed. Figure 5 results reveal that significant enhanced GPX activity in studied germplasm required more

than 36 hrs. The salt stress modulates the responses of antioxidative enzymes in leaves according to the tested germplasm and the period of stress imposition [32].The increases in GPX activities are an adaptive trait to overcome salt damage by reducing toxic levels of H_2O_2 and provide protection against oxidative stress [33], [34], [35] and [17].

Na^+ accumulation:

Different salt stresses such as 20 mM, 50 mM, 75 mM, 100 mM, 125 mM and 150 mM were given to 70 days plants grown in net house condition and after12 hrs leaf samples were collected to estimated the Na^+ accumulation in all eleven tomato germplasm (Figure 4). When the germplasms were screened at 20 mM salinity stress, the germplasm TG4, TG7, TG8, TG10 and TG11 were not accumulated significant ($P<0.01$) Na^+ content in leaves (Figure 4). After 12 hrs of salt stress except TG7 and TG8, remaining all other germplasms were accumulated significant ($P<0.01$) levels of Na^+ content at 50 mM salt stress (Figure 4). All the germplasms were accumulating significantly higher Na^+ content ($P<0.01$) when screened at 75 mM, 100 mM, 125 mM and 150 mM (Figure 4).

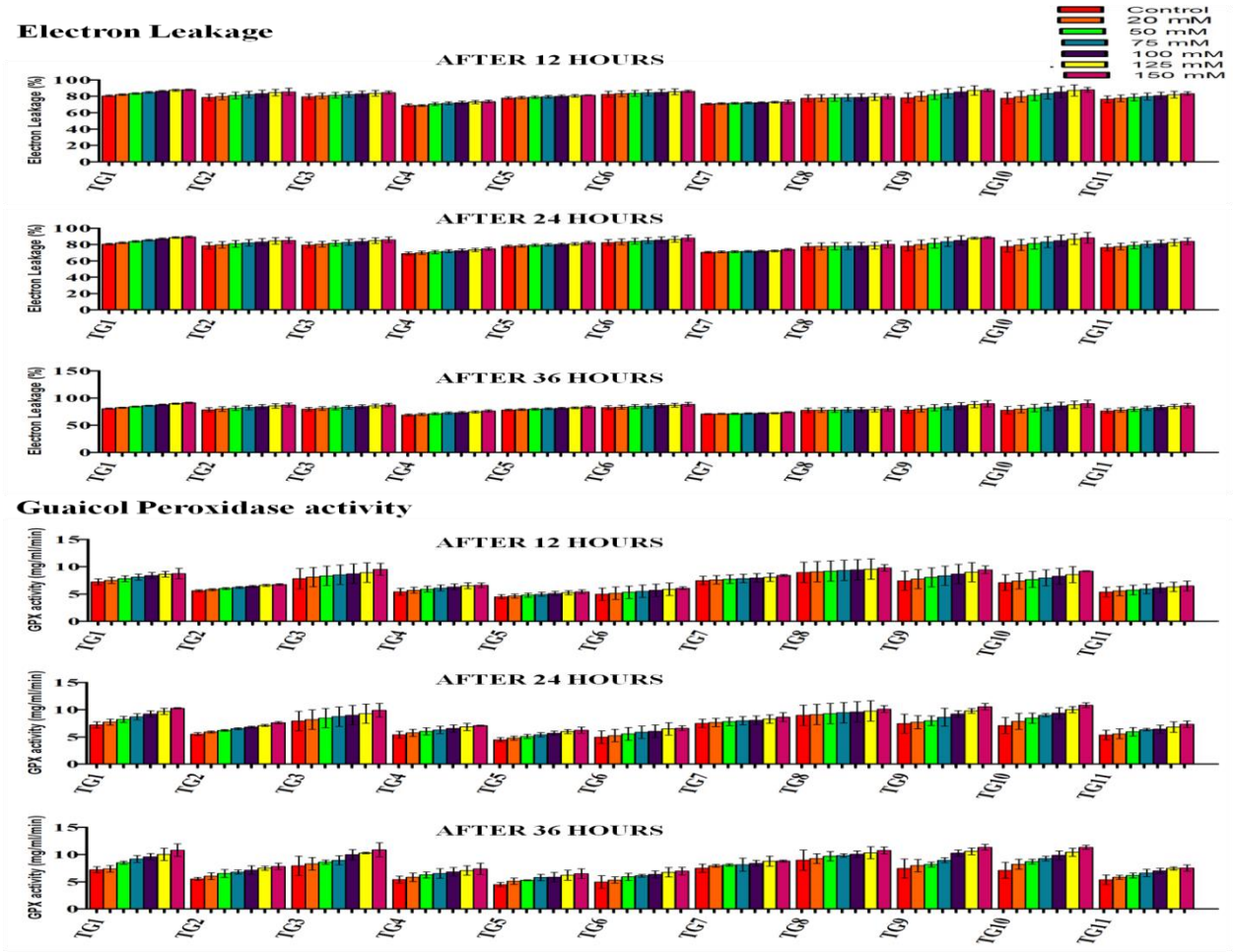

Figure 3: Effect of different time interval salinity (12, 24 & 36 hrs) on Electron Leakage and Guaicol Peroxidase activity in eleven tomato germplasm at 70 days old ontogeny stage under Net house conditions. Each bar represents the mean (± SE) of three measurements. Among all treatments statistically significant ($P\leq0.01$) with their respective controls were presented with '*' on vertical bar.

All the germplasm when treated with 20 mM salt stress all germplasm doesn't showed any significant (P<0.01) increase in their Na^+ accumulation in leaves after 24 hrs of salt stress (Figure 4). However plants screened at 50 mM salt stress except TG8 and TG11 remaining germplasm were not showed significant (P<0.01) increase in their Na^+ accumulation compared to respective controls (Figure 4). The germplasm TG4, TG5, TG6, TG9 and TG10 were not accumulated significant (P<0.01) Na^+ content at 75 mM salt stress (Figure 4). Whereas the germplasm TG5 and TG6 were not showed significantly (P<0.01) increased Na^+ accumulation even at 100 mM salt stress (Figure 4). TG7 was the only germplasm which doesn't significantly (P<0.01) increased in Na^+ accumulation at 125 mM salt stress compared to control (Figure 4). However, all germplasm were significantly (P<0.01) increased in their Na^+ accumulation at 150 mM salt stress compared to respective controls (Figure 4). Due to the effect of 36 hrs salinity incubation all germplasm not showed significant (P<0.01) changes in their Na^+ accumulation at 20 mM salt stress compared to their respective controls (Figure 4). Except the germplasm TG1, TG4 and TG10 remaining were not significantly (P<0.01) increased Na^+ levels at 50 mM salt treatment (Figure 4). At 75 mM salt stress, the germplasm TG5, TG6, TG7, TG8, TG9 and TG11 doesn't increased their Na^+ accumulation significantly (P<0.01) compared to respective controls (Figure 4). Whereas only the germplasm TG5, TG6 and TG8 were not significantly (P<0.01) increased in Na^+ content even at 100 mM salt stress (Figure 4). However the germplasm TG6 and TG8 were not showed any significant (P<0.01) increases in their Na^+ accumulation still at 150 mM salt stress compared to their respective controls (Figure 4).

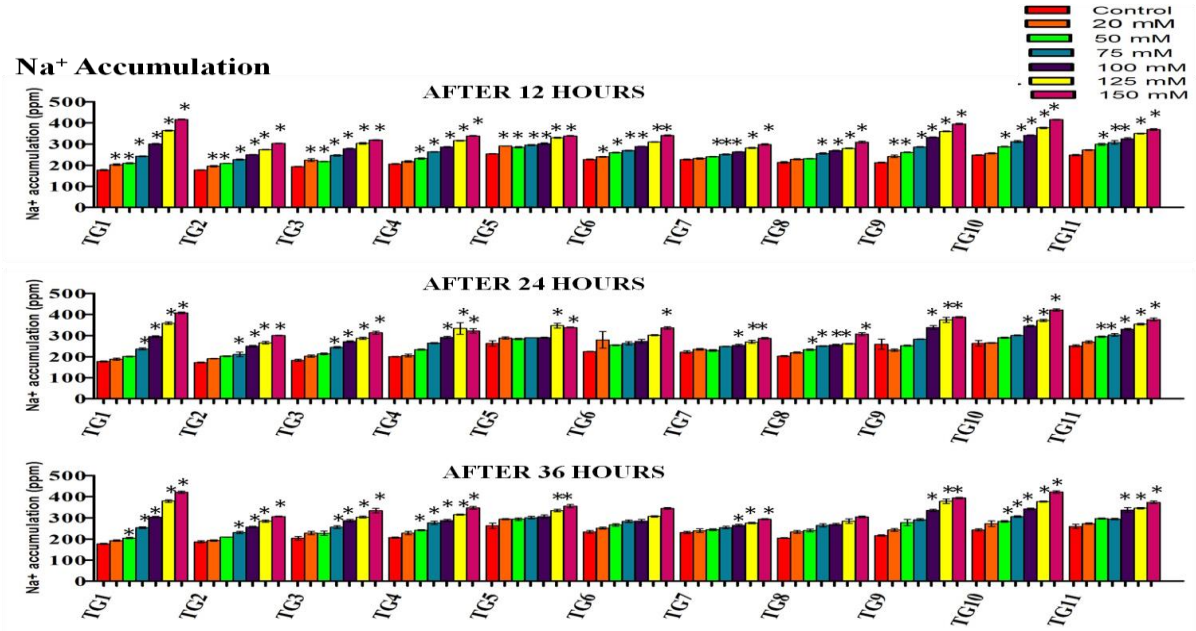

Figure 4: Effect of different time interval salinity (12, 24 & 36 hrs) on Na^+ accumulation in eleven tomato germplasm at 70 days old ontogeny stage under Net house conditions. Each bar represents the mean (± SE) of three measurements. Among all treatments statistically significant (P≤0.01) with their respective controls were presented with '*' on vertical bar.

The Na^+ influx was not statistically significant at 12 hrs of salinity stress at seedling stage as well as ontogeny stage of studied tomato germplasm (Figure 3 and Figure 6), however the influx was increased at 36 hrs of salt stress. According to [36] this is likely due to the superior Na^+ exclusion mechanism of germplasm and a peak Na^+ accumulation was occurred in at initial salt exposure (12 hrs salinity) duration comparably at 72 hrs. This finding is comparable with the way that potato cultivars respond to NaCl transport, since sensitive cultivars transport relatively more Na^+ to leaves [37].[38]Showed that among eight tested varieties, the most salt-tolerant wheat variety Kharchia 65 had the strongest root Na^+ exclusion ability. In several species, such as lupine, clover, sweet pepper, and maize, recirculation of Na^+ to roots via phloem played a role in overall salt tolerance [39]. The salt stress induces the production of ROS that is accompanied with an increase of lipid peroxidation which led to reduce membrane fluidity and selectively as well as increase of electrons leakage percentage [40], [41], [42] and [43].

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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