A Comparative Study On The Adaptability Of The Different Varieties Of Solanum Lycopersicum L. (Tomato) In Salt Stress Condition

Jobi Xavier, Joel Jose

Abstract: The objective of the present study was to study the levels of antioxidant and oxidant metabolites such as total protein, proline, peroxidase, lipid peroxidase, catalase,and anthocyanin and phenol contents in nine varieties of tomato plants (Cerasiforme (Cherry tomato), Indamrohini, Marglobe, Ns 538, Sacchariya, San Marzano 3, Suhyana, Tomato Oblate Yellow, Vanda) treated under various NaCl concentrations (50, 100, 200 and 250 Mm). Salinity is one of the significant abiotic stresses, which affects plant cell metabolism and reduces plant productivity. Plants tolerant to NaCl implement a series of adaptations to acclimate to salinity, including morphological, physiological and biochemical changes. Under saline conditions, plants have to activate different physiological and biochemical mechanisms in order to cope with the resulting stress. Such mechanisms include changes in morphology, anatomy, water relations, photosynthesis, the hormonal profile, toxic ion distribution and biochemical adaptation (such as the antioxidative metabolism response). An updated discussion on salt-induced oxidative stress and its effect on the antioxidant machinery in both salt-tolerant and salt-sensitive plants is the major part of this study. The aim of the present study is to extend our understanding of how salinity may affect the physiological characteristics of plants.

Index Terms: Anthocyanin, osmotic stress, proline, phenol, reactive oxygen species, salinity, tomato

1. INTRODUCTION

Plants are the primary producers in the ecosystem. They utilise the abiotic components in the environment such as light, water and CO_2 for the production of glucose. Plants possess some inherent characters for thriving even in adverse conditions also. About 30% of Earth's surface is covered by plants. They are also seen in a wide variety of habitats also. From the aquatic ecosystem to the desert ecosystem plants are seen growing. For thriving in a wide range of habitats, these plants have some specific mechanisms either in molecular level or morphologically. Plants are always prone to various types of stresses throughout their lifecycle. Stress can be defined as a negative factor that affects the organism which brings out a drastic change in that particular organism [1]. In the case of plants, these stresses can affect the growth and metabolic activity or even reproductive ability. These stresses may be either because of the living (biotic) factors or because of non-living (abiotic) factors. Biotic factors include insects, pests, weeds etc. Abiotic factors include light, temperature, chemicals etc. These stresses create physiological changes in the plants. They may result in the overproduction of specific secondary metabolites like phenol which are produced during the stress conditions. One type of stress that plants face is saline stress. Salinity is a major abiotic stress limiting the growth and productivity of plants in many areas of the world due to the increasing use of poor quality of water for irrigation and soil salinization. Plant adaptation or tolerance to salinity stress involves complex physiological traits, metabolic pathways, and molecular or gene networks [1]. Saline stress is defined as stress that is imparted to plants because of the presence of high salt concentration in the soil in which it is growing. The salt concentration initiates specific physiological effects on plants. They also result in morphological changes in the plants. Salinity is significant stress limiting the increase in the demand for food crops. More than 20% of cultivated land worldwide (~ about 45 hectares) is affected by salt stress and the amount is increasing day by day. Plants on the basis of adaptive evolution can be classified roughly into two major types: the halophytes (that can withstand salinity) and the glycophytes (that cannot withstand salinity and eventually die) [2], [3]. Salinity stress involves changes in various physiological and metabolic processes, depending on the severity and duration of the stress, and ultimately inhibits crop production [4]. During the initial phases of salinity stress, the water absorption capacity of root systems decreases and water loss from leaves is accelerated due to osmotic stress of high salt accumulation in soil and plants, and therefore salinity stress is also considered as hyperosmotic stress [5]. Osmotic stress in the initial stage of salinity stress causes various physiological changes, such as interruption of membranes, nutrient imbalance, impairs the ability to detoxify reactive oxygen species (ROS), differences in the antioxidant enzymes and decreased photosynthetic activity, and decrease in stomatal aperture[6]. One of the most detrimental effects of salinity stress is the accumulation of Na+ and Cl− ions in tissues of plants exposed to soils with high NaCl concentrations. The entry of both Na+ and Cl− into the cells causes severe ion imbalance and excess uptake might cause significant physiological disorder(s). High Na+ concentration inhibits the uptake of K+ ions which is an essential element for growth and development that results in lower productivity and may even lead to death [6]. Low molecular mass compounds known as compatible solutes is accumulated under salt stress. These compatible solutes include proline, glycine betaine, sugars, proteins, polyols, etc. They do not interfere with the normal biochemical reactions and helps the plants in building resistance against the stress [7].Among the various sources of soil salinity, irrigation combined with poor drainage is the most serious, because it represents losses of once productive agricultural land [8].When the water evaporates, Ca^{2+} and Mg^{2+} often precipitate into carbonates, leaving Na^{+} dominant in

- *Jobi Xavier is currently working as Assistant Professor in the department of Life Sciences, CHRIST (Deemed to be University) Bengaluru, Karnataka, India PH-9916929979. E-mail: frjobi.xavier@christuniversity.in*
- *Joel Jose is currently working as Teaching Associate in the department of Life Sciences, CHRIST (Deemed to be University) Bengaluru, Karnataka, India PH-8792175538 E-mail: joel.jose@christuniversity.in*

the soil [9]. The present study is an attempt to understand the adaptability of the different varieties of Solanum lycopersicum (tomato) in salt stress condition. The most adaptable tomato plants can be used by the agriculture in salt dominated soils. The study was aimed at supporting the farmers to choose the salt-resistant varieties of tomato plants. Nine varieties of tomato plants namely, Cerasiforme (Cherry tomato), Indamrohini, Marglobe, Ns 538, Sacchariya, San Marzano, Suhyana, Tomato Oblate Yellow, Vanda were selected in the present study and focussed on the productivity of salt-resistant varieties.

2. MATERIALS AND METHODS

Collection of Samples

The seeds collected from Lalbagh Botanical Gardens, Bangalore, Karnataka. It is a public botanical garden in southern Bengaluru, India. Nine varieties of seeds of Solanum lycopersicum, (tomato) were collected. The nine varieties were Cerasiforme (Cherry tomato), Indian Rohini, Marglobe, NS 538, Sachriya , San Marzano 3, Suhyanu, Tomato Oblate Yellow, Vanda.

Growth of plants

Different varieties of seeds were sown in different poly-fibre trays which were filled with damp coco-peat, and the trays were labelled accordingly. The seeds were watered every day to maintain the moisture in the coco-peat. After the seeds germinated, on the 15th day, when the seedlings reached a sufficient height, they were transplanted into pots filled with red soil and were watered. 4-5 seedlings were planted in each pot with a sufficient amount of space between the seedlings.

Preparation of NaCl

The plants were subjected to NaCl treatments. Five different concentration of NaCl; 50mM, 100mM, 150mM, 200mM, 250mM, were chosen for the experiment along with a control without any NaCl treatment. A total of 16 pots were maintained for each variety where one pot was kept as control, and three pots were kept for each concentration of NaCl treatment, i.e., 50mM, 100 Mm, 150mM, 200mM, and 250mM of NaCl solution respectively. The plants were provided with different concentrations of NaCl solution every alternate day, and the rest of the days they were watered with tap water. A gradual increase in NaCl concentration was provided in order to prevent osmotic shock. This was continued to for 4 weeks. Protein estimation by Bradford Method: Various concentrations of working standard (BSA) with the concentration of 100µg/ml were pipetted into a series of test-tubes.5mg of the sample was also added to respective test-tubes. The volume of all the test tubes was made to 1 ml with distilled water, a tube with 1ml of water served as blank. 5ml of Bradford reagent was added to all the test tubes including the blank. The contents were mixed by shaking the tubes and were incubated at room temperature for 10mins, and the absorbance was checked at 595nm. [10]

Phenol estimation:

1g of the sample was mixed with 10 ml of methanol using mortar and pestle and was transferred to a centrifuge tube and kept in a shaker for 48 hours at 30℃, and then filtered to remove insoluble material. The extract was concentrated to dryness, and the solid residue was then dissolved in the same solvent. The total phenolic content of the extract was determined by Folin-coicaltau reagent [11]. Various concentrations of working standard from a solution containing 100 µg of catechol were taken into test tubes and were made up to 3 ml using distilled water. 200 µl of crude extract (1mg/ml) was made up to 3ml with distilled water, followed by 0.5 ml of Folin-coicaltau reagent (10 times diluted) to all test tubes and kept for 3min, followed by addition of 2ml of 20% sodium carbonate to all test-tubes. A blank was also maintained and these test tubes were placed in a water bath for 1 minute and absorbance was checked at 638nm. [11]

Proline estimation

0.5g of the plant material was homogenised in 10 ml of 3% aqueous sulpho-salicylic acid, and this homogenate was filtered through whatman's filter paper. 2.2ml of the filtrate was taken in a test-tube, and 2ml of glacial acetic acid and 2ml of acid ninhydrin were added. This was kept in boiling water bath for one hour. The reaction was terminated by placing the tube in an ice bath. 4ml of toluene was added to the reaction mixture and was stirred for 20-30 seconds. Toluene layer was separated and the red colour intensity was measured at 520nm nm using toluene as reference. The proline concentration was determined using a standard concentration curve [12].

Purification of peroxidase

Protein Estimation - 0.5 g of each seed variety was taken and ground with 3 ml of NaOH drop by drop using a pestle and mortar. Then it was centrifuged for 15 minutes at 10,000 rpm. After centrifugation, the supernatant was taken out and stored. Protein was estimated by the Bradford method. Enzyme activity of Peroxidase- For the assay of peroxidase, Guaiacol is used as substrate. Guaiacol + H_2O_2 Oxidised guaiacol + $2H_2O$. 1g of seeds was mixed with 3ml of 0.1 M phosphate buffer of pH 7.0 and ground in a pre-cooled pestle and mortar. The homogenate was centrifuged at 18,000 rpm at 5℃ for 15 minutes. The supernatant was used as an enzyme source within 2-4 hours. 3ml buffer solution, 0.05ml guaiacol solution, 0.1ml enzyme extract and 0.03ml hydrogen peroxidase solution was taken in a cuvette. It was mixed well and the absorbance was measured using a spectrophotometer at 436 nm. A stopwatch was started after the absorbance reached 0.05 and the time taken in minutes for the absorbance (Δt) to increase 0.1 was noted down. Since the extinction co-efficient of guaiacol dehydrogenation product at 436nm under the condition specified is 6.39 per micromole, the enzyme activity per litre of the extract is calculated as below [13].

$$\text{Enzyme activity (units/litre)} = \frac{3.18 \times 0.1 \times 1000}{6.39 \times 1 \times \Delta t \times 0.1} = \frac{500}{\Delta t}.$$

Lipid peroxidase

Tomato leaves, mortar, pestle, muslin cloth, 0.1% Trichloroacetic acid (TCA), 20 % Trichloroacetic acid containing 0.5% thiobarbituric acid (TBA) were used in this experiment. The amount of Lipid Peroxidase present in each of the leaf tissue was measured in terms of malondialdehyde (MDA) content which was estimated by Sairam and Saxena's method [14]. 0.5g of each leaf sample was weighed and ground using a mortar and pestle in 5ml of 0.1 % trichloroacetic acid (TCA). The ground sample was then centrifuged for 5 minutes at 10,000g. 1ml of the supernatant was taken in a test tube and to this, 4ml of 20% v/v TCA

containing 0.5% v/v thiobarbituric acid (TBA) was added. The mixture was then heated for 30 minutes at 95 ºC and then cooled on ice. It was then centrifuged again for 5 minutes at 10,000g. The supernatant was taken and the absorbance was measured at 532 and 600nm. The amount of MDA present in each of the leaf sample was expressed as nmol g^{-1} FW using the coefficient of absorbance of 15 mmol l^{-1} cm^{-1}.

Anthocyanin

Estimation of anthocyanin content in the leaf samples was determined by Mancinelli method [15]. 1g of each of the leaf samples were weighed and ground in a mortar and pestle using 3ml of methanol-HCl (1% HCL, v/v) The samples were placed in the fridge for 48hours at 4 ºC . After 48 hours, the extracts were filtered through two layers of muslin cloth. The residue was discarded and the filtrate was used to measure the anthocyanin content in a spectrophotometer at 530 and 657 nm wavelengths. The results were calculated using the formula:

$\Delta A_{(530-657)}\, g^{-1}FW$.

3. RESULTS

Estimation of Protein

Nine varieties of tomato plants were used for the study. In Cerasiforme variety the highest value of protein was found in 150Mm and the lowest was in 50mM. The protein content was found to be 4884.7, 3570, 4462.9, 7406.7, 4850.3, 3833.2 µg per gram of sample for control, 50Mm, 100Mm, 150mM, 200Mm, 250Mm respectively. The highest concentration of protein in Indhamrohini was found in control and the lowest was 3696 in 200mM. The concentration of protein obtained in different varieties of tomato treated with different concentrations of NaCl is given below (Table 1). It was observed that as the concentration of NaCl increased the amount of protein also increased. It was also observed that in the concentrations of 200 mM and 250mM of NaCl, the quantity of protein was declined which clearly pointed out that the varieties were not well adapted to the high concentrations of salt. But Indhamrohini and ns538 varieties were found to be tolerant to the salt stress. The ns538 variety treated with 250mM NaCl has produced more protein than its control.

TABLE 1

Protein concentration in mg/g of samples of different varieties of Solanum lycopersicum grown in different concentrations of NaCl

Varieties	control	50 mM	100 mM	150 mM	200 mM	250 mM
Cerasi-forme	4.89	3.57	4.46	7.41	4.85	3.83
Indham rohini	5.73	4.84	4.93	3.95	3.69	4.70
marglobe	5.59	6.27	5.76	4.26	3.12	2.49
ns538	6.31	7.27	5.96	4.31	3.23	6.38
sachriya	0.58	0.76	0.52	0.97	0.53	0.50
san-marzano	8.16	5.52	6.14	8.27	7.42	5.53
suhyana	3.08	0.16	1.16	1.25	2.90	2.88
oblate yellow	0.66	0.57	0.78	0.38	0.40	0.41
vanda	0.79	0.66	0.69	0.54	0.46	0.50

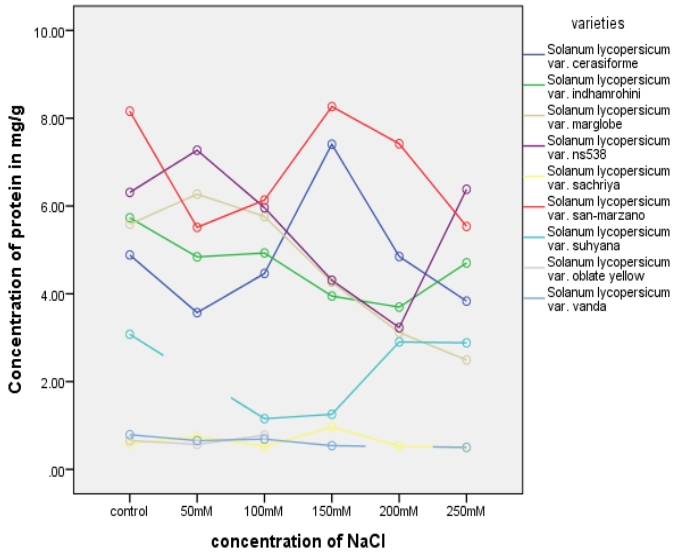

Fig.1*. Concentration of protein in mg/g of the plant samples of different varieties of Solanum lycopersicum*

Phenol Estimation

In the present study, the total phenol concentration was increased significantly in all the varieties. The varieties exhibited increased phenol concentrations in response to the dose of 100Mm and above. Most of them exhibited high phenol concentrations in 150Mm. In Cerasiforme (cherry tomato), the total phenol content was observed high in 150mM concentration (550µg/g). And the total phenolic content was less in 50mM concentration (150µg/g). The other concentration such as 100, 200,250mM and control showed 300, 350, 400 and 450µg/g respectively. In Indhamrohini, the total phenol content was observed high in 250mM concentration (633.3µg/g). And the total phenol content was low in control concentration (206.3µg/g). The other concentration such as 50, 100, 150, 200mM showed 469.8, 277.7, 477.7 and 522.2µg/g respectively. In Marglobe variety total phenol content was observed high in 250mM concentration (531µg/g). Total phenol content was less in control concentration (187.5µg/g). The other concentration such as 50, 100, 150, 200 mM showed 312.3, 250, 343 and 500µg/g respectively. In NS538 variety total phenol content was observed high in 200mM concentration (650µg/g). Total phenol content was less in 50mM concentration (125µg/g). The other concentration such as 100, 150, 250mM and control showed 350, 400, 450 and 350µg/g respectively. In Sachariya variety total phenol content was observed high in 150mM concentration (270µg/g). Total phenol content was less in 50mM concentration (90µg/g). The other concentration such as 100, 200, 250mM and control showed 150, 270, 120 and 190µg/ml respectively. In San Marzano variety, total phenol content was observed high in 250mM concentration

(1000µg/ml). Total phenol content was less in 100mM concentration (150µg/ml). In Suhyana variety total phenol content was observed high in 100mM concentration (975µg/g). Total phenol content was less in control concentration (100µg/g). The other concentration such as 50,150, 200, and 250mM showed 461, 375, and 451 and 556µg/g respectively. In tomato oblate yellow variety total phenol content was observed high in 250mM concentration (180µg/g). Total phenol content was less in control concentration (100µg/g). In Vanda variety total phenol content was observed high in 250mM concentration (370µg/g). Total phenol content was less in 150mM concentration (250µg/g).

TABLE 2

Phenol concentrations in mg/g of samples of different varieties of Solanum lycopersicum grown in different concentrations of NaCl

Varieties	control	50 mM	100 mM	150 mM	200 mM	250 mM
Cerasi-forme	0.45	0.15	0.30	0.55	0.35	0.40
Indham rohini	0.21	0.47	0.28	0.48	0.52	0.63
Marglobe	0.19	0.31	0.25	0.34	0.50	0.53
ns538	0.35	0.13	0.35	0.40	0.65	0.45
Sachariya	0.19	0.09	0.15	0.27	0.16	0.12
San-marzano	0.50	0.21	0.115	0.38	0.45	1.00
suhyana	0.10	0.46	0.98	0.38	0.45	0.56
oblate yellow	0.10	0.13	0.15	0.11	0.12	0.18
vanda	0.30	0.26	0.33	0.25	0.30	0.37

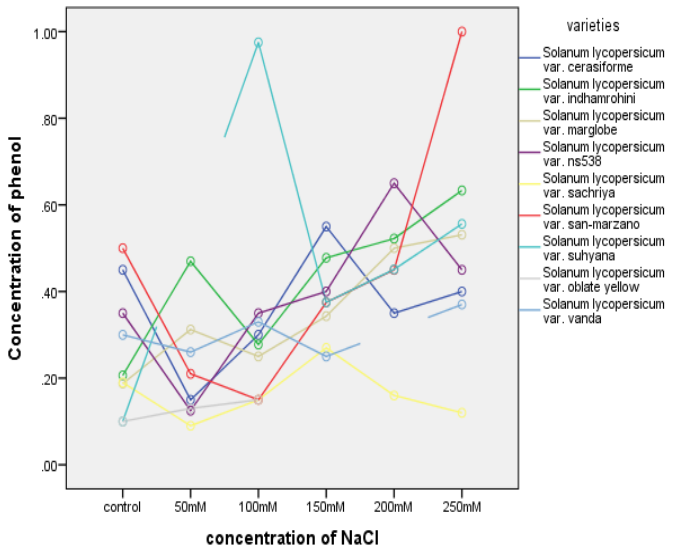

Fig.2*. Concentration of Phenol in mg/g of the plant samples of different varieties of Solanum lycopersicum*

Proline estimation

The amount of proline for nine varieties of tomato treated with five different concentrations of sodium chloride clearly showed the adaptability of those varieties to the stress conditions. In variety Cherry tomato the highest amount of proline was found to be in 200 mM concentration of sodium chloride and the least amount of proline concentration was found to be in 100 mM concentration of sodium chloride. But in the case of cherry tomato in contrary to other varieties the control plant without any stress has a higher concentration of proline than that found in 50 mM, 100 mM, 150 mM, and 250 mM. We may infer from that the amount of proline is high in the variety of cherry tomato without inducing any stress and also in cherry tomato variety maximum amount of salt stress was exhibited in 200 mM concentration. In Indhamrohini highest proline content was observed in 250 mM (630 µg/g) and the lowest was observed in control (230 µg/g). In Marglobe highest proline content was observed in 250 mM (639.5 µg/g) and lowest was observed in 100 mM (112.5 µg/g). Though the amount of proline in control is higher than 50 mM, 100 mM from which we can infer that this variety naturally produces some amount of proline and a decrease in the proline content in 50 mM, and 100 mM may be due to the treatment of sodium chloride. In NS 538 highest proline content was observed in 250 mM (609 µg/g) and lowest was observed in control (201 µg/g). In Sachariya highest proline content was observed in 250 mM (610 µg/g) and the lowest was observed in control (190 µg/g). In san marzano highest proline content was observed in 250 mM (800 µg/g) and lowest was observed in control (180 µg/g). In Suhyana highest proline content was observed in 250 mM (380 µg/g) and the lowest was observed in control (180 µg/g). In tomato oblate yellow highest proline content was observed in 200 mM (450 µg/g) and lowest was observed in 250 mM (50 µg/g).In this variety, there is a drastic change from most of the other varieties like in the case of cherry tomato the highest concentration of proline was found in 200 but the least amount of proline was found in 250. In Vanda highest proline content was observed in 250 mM (700 µg/g), and the lowest was observed in control (280 µg/g).

TABLE 3

Proline concentrations in mg/g samples of different varieties of Solanum lycopersicum grown in different concentrations of NaCl

Varieties	control	50mM	100mM	150mM	200mM	250mM
Cerasi-forme	0.46	0.18	0.17	0.20	0.67	0.24
Indham rohini	0.23	0.40	0.37	0.34	0.35	0.63
Marglobe	0.23	0.13	0.11	0.24	0.63	0.64
ns538	0.20	0.21	0.20	0.31	0.50	0.61
Sachariya	0.19	0.20	0.30	0.36	0.50	0.61
san-marzano	0.18	0.40	0.50	0.55	0.60	0.80
suhyana	0.18	0.20	0.26	0.28	0.30	0.38
oblate yellow	0.20	0.08	0.30	0.40	0.45	0.05

vanda	0.28	0.50	0.55	0.57	0.625	0.70

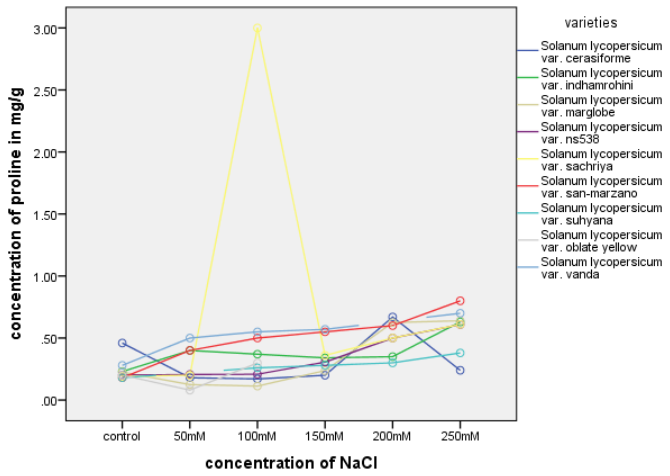

Fig.3*. Concentration of proline in mg/g of the plant samples of different varieties of Solanum lycopersicum*

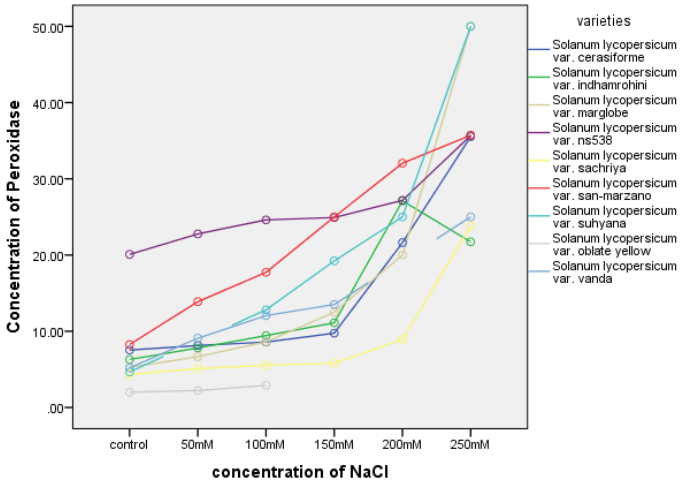

Fig.4*. Concentration of peroxidase of the plant samples of different varieties of Solanum lycopersicum*

Estimation of Peroxidase

All the varieties have shown high concentrations of peroxidase activity in the 250mM salt stress, except the variety Indhamrohini (21.73). Cherry tomato showed a sudden increase in the peroxidase concentration at 200mM (21.63) and continued to increase in 250mM (35.54) as well. Marglobe variety showed a slow and high change in the 250mM (50). The ns538 variety had the highest concentration of the peroxidase activity among all the nine varieties. Sachariya showed a slow increase in peak but having a high concentration in 250mM (23.8) San Marzano showed a gradual increase in the level of peroxidase. Oblate yellow showed the least amount of peroxidase in the entire nine varieties.

TABLE 4

Peroxidase concentrations in nmol per litre samples of different varieties of Solanum lycopersicum grown in different concentrations of NaCl

Varieties	control	50 mM	100 mM	150 mM	200 mM	250 mM
Cerasi-forme	7.54	8.13	8.58	9.75	21.63	35.54
Indham rohini	6.30	7.81	9.43	11.11	27.11	21.73
marglobe	5.26	6.66	8.62	12.5	20.00	50.00
ns538	20.08	22.77	24.61	24.92	27.18	35.68
sachriya	4.34	5.10	5.55	5.81	8.92	23.80
san-marzano	8.26	13.88	17.73	25	32.05	35.71
suhyana	4.67	10.64	12.82	19.23	25.00	50.00
oblate yellow	2.00	2.23	2.90	5.00	6.25	7.93
vanda	5.20	9.09	12.05	13.51	20	25.00

Estimation of Lipid peroxidise

The different varieties of tomato showed varying levels of production of lipid peroxidase with respect to different salt concentrations. This indicates that different varieties have a different level of resistance to salt stress. In the case of Cerasiforme (cherry tomato), the MDA content stayed relatively in the same range for control 50mM, 100mM and 150mM. This indicated that the plant was capable of tolerating NaCl stress up to 150mM concentration and was able to maintain its vegetative growth and metabolism at these concentrations. In the case of Indhamrohini, the MDA levels were low in control and 50mM NaCl concentrations. The level of MDA then increased drastically at 100mM concentration and the levels were maintained even in 150mM concentration indicating that the variety was capable of withstanding salt stress till 150mM salt stress. The values of MDA then reduced consistently for 200mM and 250mM accompanied by stunted growth indicating the incapability of the variety to tolerate such levels of stress. In the case of Marglobe variety, the MDA levels were low in control and increased drastically at 50mM indicating that the variety perceived even such low concentrations as stress and increased the lipid peroxidase activity to overcome it. The MDA levels kept constantly decreasing thereafter as the plant became more and more incapable of tolerating the stress. In the case of ns 538, the MDA levels were found to increase at 50mM concentration from the levels of the control. The MDA levels were then seen to decrease steadily till 150mM. Then there was an increase in the 200mM concentration and then decreased at 250mM. In the case of Sachariya, the MDA levels constantly increased from control to 150mM concentration and then it decreased at 200mM and increased again slightly at 250mM. A very different result was obtained for San Marzano; the MDA levels were high for control and decreased only slightly for 50mM. In the case of Oblate Yellow variety, there was a slight decrease observed from control to 50mM indicating that this concentration of salt was optimal for its growth. In the case of Suhyana, the MDA levels increased slightly from control to 50mM and remained constant until 150mM. Similar results

were observed for the tomato variety Vanda; the MDA levels remained more or less constant for all the different salt concentrations of salt stress. There was a slight increase from control to 50mM. This was followed by slight decrease at 100mM, 150mM and 200mM and then followed by a slight increase.

TABLE 5

Lipid Peroxidase concentrations in nmol per gram samples of different varieties of Solanum lycopersicum grown in different concentrations of NaCl

Varieties	control	50 mM	100 mM	150 mM	200 mM	250 mM
Cerasi-forme	3.22	4.51	3.48	3.22	9.41	1.8
Indham rohini	1.29	0.99	2.56	2.54	1.39	0.74
marglobe	0.25	1.7	1.25	0.76	0.4	0.77
ns538	1.16	1.78	1.12	0.89	1.85	0.94
sachriya	0.29	1.45	2.96	4.73	1.94	3.18
san-marzano	1.97	1.52	0.33	0.63	1.18	1.08
suhyana	4.7	5.44	5.2	5.22	3.53	3.74
oblate yellow	3.6	1.2	4.9	15.2	10.3	20.6
vanda	14.4	18	17.2	15.4	12.6	16

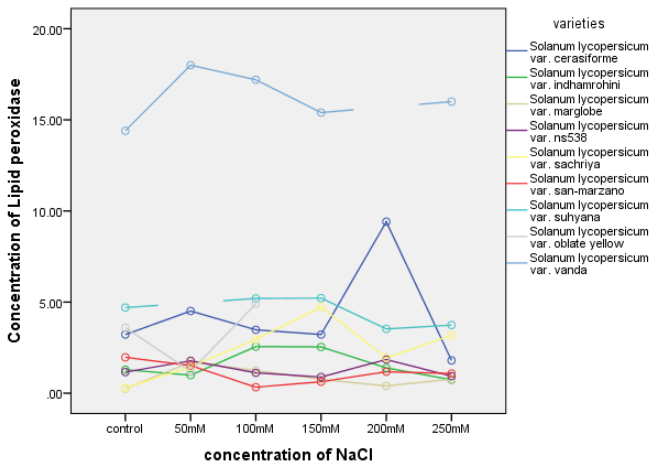

Fig.5*. Concentration of Lipid peroxidase in mmole/g of the plant samples of different varieties of Solanum lycopersicum*

Estimation of Anthocyanin

In the present study, the estimation of anthocyanin for nine varieties of tomato was conducted. Each verity had control, and triplicates of the nine different varieties which were treated with five different concentrations of sodium chloride, i.e., 50 mM, 100 mM, 150 mM, 200 mM, and 250 mM showed high anthocyanin content. The anthocyanin content for cherry tomato was estimated and it was found that the maximum anthocyanin was obtained for Control [0.008 g^{-1} FW] and the minimum was obtained for both 200 and 250 mM [0.001 g^{-1} FW]. The anthocyanin content for Indam Rohini variety was found and the maximum amount of anthocyanin was obtained for 150 mM [0.055 g^{-1} FW] and the least amount was obtained for 50mM [0.007g $^{-1}$FW]. In Marglobe variety, the maximum amount of anthocyanin was found to be in 100 mM [0.007 g^{-1}FW] and the minimum was found to be in 150 mM [0.001 g^{-1} FW]. In Indam- rohini and Marglobe variety, it did not follow the prevailing trend where there was a fluctuation in the pattern in anthocyanin accumulation. In ns538, the maximum amount of anthocyanin was observed in two concentrations i.e., 200 and 250 mM. Similarly, the minimum amount of anthocyanin was observed in two concentration of control and 50 mM. In Sachariya variety, the maximum amount of anthocyanin content was obtained for the 100 mM concentration [0.006 g^{-1}FW] and the minimum was obtained for both 50- and 200-mM concentrations [0.001g $^{-1}$FW]. This also showed a varied fluctuation in anthocyanin accumulation than the pattern like the pattern exhibited by the varieties Indhamrohini and Marglobe. In San Marzano 3 variety the maximum amount of anthocyanin was found to be in 150 mM [0.01 g $^{-1}$FW] and the minimum was found to be in 250 and 200 mM concentrations [0.001 g $^{-1}$ FW]. For the Suhyana variety, the maximum amount of anthocyanin content was obtained for the 50 mM concentration [0.01 g^{-1}FW] and the minimum was obtained for both 150 and 200 mM concentrations [0.003g $^{-1}$FW]. For the Tomato Oblate Yellow variety, the maximum amount of anthocyanin content obtained for the 150 mM concentration [0.236 g^{-1}FW] and the minimum was obtained for 250 mM concentration [0.032 g $^{-1}$FW].

TABLE 6

Anthocyanin concentrations in g-1 FW samples of different varieties of Solanum lycopersicum grown in different concentrations of NaCl

Varieties	control	50mM	100 mM	150 mM	200 mM	250 mM
Cerasi-forme	0.008	0.007	0.003	0.005	0.001	0.001
Indham rohini	0.008	0.007	0.012	0.055	0.042	0.051
marglobe	0.001	0.003	0.007	0.001	0.005	0.002
ns538	0.001	0.001	0.003	0.005	0.01	0.01
sachriya	0.005	0.001	0.006	0.003	0.001	0.002
san-marzano	0.003	0.001	0.004	0.01	0.001	0.001
suhyana	0.001	0.01	0.006	0.003	0.003	0.007
oblate yellow	0.191	0.186	0.077	0.236	0.16	0.032
vanda	0.224	0.279	0.267	0.232	0.196	0.248

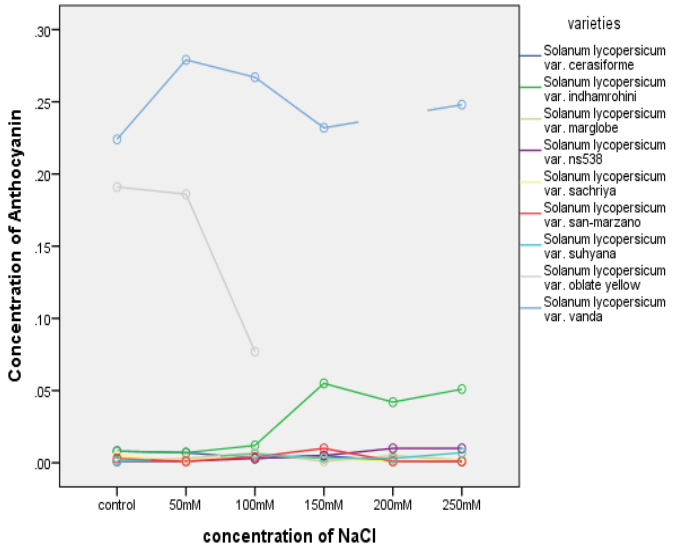

***Fig.6**. Concentration of Anthocyanin of the plant samples of different varieties of Solanum lycopersicum*

Statistical Analysis

TABLE 7.
ANOVA Statistics of the significance of metabolites on different Varieties

Model	F	Sig.
Dependent Variable: Protein Predictors: (Constant), varieties	1.377	.242[a]
Dependent Variable: phenol Predictors: (Constant), varieties	.001	.976[a]
Dependent Variable: proline Predictors: (Constant), varieties	5.575	.019[b]
Dependent Variable: peroxidase Predictors: (Constant), varieties	8.656	.004[b]
Dependent Variable: Lipidperoxidase Predictors: (Constant), varieties	6.153	.014[b]
Dependent Variable: Anthocyanin Predictors: (Constant), varieties	21.066	.000[b]

a= P>0.05, which implies that there is a statistically significant difference. B=P<0.05 which implies that there is no statistically significant difference.

TABLE 8
ANOVA Statistics of Significance of metabolites on Concentrations of NaCl

Model	F	Sig.
Dependent Variable: Protein Predictors: (Constant), concentration	7.032	.009[b]
Dependent Variable: phenol Predictors: (Constant), concentration	73.665	.000[b]
Dependent Variable: proline Predictors: (Constant), concentration	62.350	.000[b]
Dependent Variable: peroxidase Predictors: (Constant), Concentration	225.411	.000[b]
Dependent Variable: Lipidperoxidase Predictors: (Constant), concentration	.276	.600[a]
Dependent Variable: Anthocyanin Predictors: (Constant), concentration	.378	.540[a]

a= P>0.05, which implies that there is a statistically significant difference. B=P<0.05 which implies that there is no statistically significant difference.

4. DISCUSSION

Salinity is an essential factor which affects plant growth and it can cause drastic changes in plant metabolism and can cause modifications in plant growth development and gene expression. This type of modifications results in the accumulation or depletion of specific proteins and other compounds which could increase, decrease appear or disappear after salt treatment. There are many reports suggested showing that the protein pattern changes are accompanied by the biological changes in the adaptation process, which makes the organism more fit in the altered environment. According to the earlier studies conducted salt stress induces quantitative and qualitative changes in protein content of the plant cells [16]. Higher content of soluble proteins has also been reported in salt-tolerant cultivars of barley, sunflower etc. According to our results, we came to the conclusion that increasing or decreasing protein contents in plants exposed to salt stress are relatively genotype-dependent. When tomato plants were treated with salt one of the up-regulated proteins was a salt tolerant protein with a mol wt. of 25 kDa. It has been already reported that salt and osmotic stresses can increase the expression of stress proteins [16]. In response to abiotic stress, plants have developed a wide variety of highly efficient mechanisms to sense, respond and adapt to a wide range of environmental changes. A typical defensive activated in plants exposed to stressing plants is the production and accumulation of phenolic compounds. Some enzymes involved in the phenolic metabolism such as polyphenol oxidase and peroxidase generally respond actively to the presence of stress in the plant. In the present study, the total phenol concentration was increased significantly in all the varieties. The increase in antioxidant phenolic compounds levels in leaves can be

considered as part of the response induced to cope with oxidative stress induced by salinity. Thus, salt-stressed plants might represent potential sources of polyphenols, by increasing polyphenol concentration in the tissues, which is an issue that is directly tied with human health since these compounds are known to be bioactive compounds [17]. Proline protects the plants from various stresses and also helps plants to recover from stress more rapidly. When applied exogenously to plants exposed to stress, Proline resulted in the increased growth and other physiological characteristics of plants. When plants are exposed to stressful environmental conditions, the production of Reactive Oxygen Species (ROS) increases and can cause significant damage to the cells. Antioxidant defences, which can detoxify ROS, are present in plants. A major hydrogen peroxide detoxifying system in plant cells is the ascorbate-glutathione cycle, in which, ascorbate peroxidase (APX) enzymes play a key role in catalyzing the conversion of H_2O_2 into H_2O, using ascorbate as a specific electron donor. Different APX isoforms are present in distinct subcellular compartments, such as chloroplasts, mitochondria, peroxisome, and cytosol. The expression of APX genes is regulated in response to biotic and abiotic stresses as well as during plant development. The APX responses are directly involved in the protection of plant cells against adverse environmental conditions. Furthermore, mutant plants APX genes showed alterations in growth, physiology and antioxidant metabolism revealing those enzymes involved in the typical plant development. Tanaka et al. [18] analyzed and studied the overexpression of yeast mitochondrial Mn-SOD in transgenic rice plants. Under salt stress, levels of Zn-SOD and catalase decreased in both the transformants and the wild type of rice, but in the transformant rice, the levels of total SOD were maintained at a high level and those of APX increased when compared to the wild type. This might correlate with the protection of the photosynthetic system against salt stress-induced damage. Most of the major crop plants are sensitive to variations in environmental factors salt stress being an important one. Plants can naturally tolerate increased salt concentrations up to a level beyond which their vegetative growth and yield are affected [19], [20], [21]. Tomatoes are found to be moderately resistant to salt, withstanding a soil ECsat up to 6 dS m^{-1}, so that it can be cultivated in regions exposed to a certain degree of soil salinization [22].

5. CONCLUSION

The best salt tolerance was observed in the tomato varieties that had high levels of MDA in their control plants, that is they produce salt stress counteracting molecules naturally. This indicates that these varieties, namely Cerasiforme (Cherry tomato), Suhyana and Vanda, are capable of being cultivated in highly saline soil conditions and are most capable of healthy metabolism, growth and yield in such environmental stress conditions. It is found that the anthocyanin content is comparatively higher in plants that are grown in stressful conditions than the one which does not experience stress as anthocyanin metabolism is increased as a mechanism to tide over the environmental stress.

Authors' Contributions

All the listed authors have made a significant scientific contribution to the research in the manuscript. Both authors performed the experiment, analysed the data, and wrote the manuscript.

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REFERENCES

[1] J.A Hernandez, A. Jiménez, P. Mullineaux and F.Sevilla, "Tolerance of pea ´ (Pisum sativum L.) to long-term salt stress is associated with induction of antioxidant defenses." in Plant Cell Environ, 23, pp:853–862, 2000.

[2] T. Flowers, "Improving crop salt tolerance", Journal of Experimental Botany, vol. 55, no. 396, pp. 307-319, 2004.

[3] R. Munns and M. Tester, "Mechanisms of Salinity Tolerance", Annual Review of Plant Biology, vol. 59, no. 1, pp. 651-681, 2008

[4] R. James, C. Blake, C. Byrt and R. Munns, "Major genes for Na+ exclusion, Nax1 and Nax2 (wheat HKT1;4 and HKT1;5), decrease Na+ accumulation in bread wheat leaves under saline and waterlogged conditions", Journal of Experimental Botany, vol. 62, no. 8, pp. 2939-2947, 2011

[5] R. Munns, "Genes and salt tolerance: bringing them together", New Phytologist, vol. 167, no. 3, pp. 645-663, 2005

[6] A. Rahnama, R. James, K. Poustini and R. Munns, "Stomatal conductance as a screen for osmotic stress tolerance in durum wheat growing in saline soil", Functional Plant Biology, vol. 37, no. 3, p. 255, 2010.

[7] S. Rasool, A. Hameed, M. Azooz, Muneeb-u-Rehman, T. Siddiqi and P. Ahmad, "Salt Stress: Causes, Types and Responses of Plants", Ecophysiology and Responses of Plants under Salt Stress, pp. 1-24, 2012.

[8] P. Carillo, M. Grazia, G. Pontecorvo, A. Fuggi and P. Woodrow, "Salinity Stress and Salt Tolerance", Abiotic Stress in Plants - Mechanisms and Adaptations, 2011.

[9] R. Serrano, F. Culiañz-Maciá and V. Moreno, "Genetic engineering of salt and drought tolerance with yeast regulatory genes", Scientia Horticulturae, vol. 78, no. 1-4, pp. 261-269, 1998.

[10] Bradford, M. M, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding", Anal Biochem, vol.72, pp. 248-254. 1976.

[11] Jyoti Jeena James, Dylan D. Silva, Saji Varghese, Jobi Xavier and Kuppusamy Alagesan Paari, Drinking straw from coconut leaf: A study of its epicuticular wax content and phenol extrusion properties. Asian J. Plant Sci., vol. 18, no.3, pp.139-147. 2019.

[12] E. Abhraham and C. Cabassa, "Methods for Determination of Proline in Plants", Methods in molecular biology, vol. 639, pp. 317-331, 2010.

[13] L. Rebecca, S. Sharmila and E. Kowsalya, "Partial purification of peroxidase from marine algae", Der Pharmacia Lettre, vol. 8, no. 352-354, 2016.

[14] M. Khan and N. Khan, Reactive Oxygen Species and Antioxidant Systems in Plants: Role and Regulation under Abiotic Stress. Singapore: Springer, 2017.

[15] F. Amini, A. A. Ehsanpour, Q. T. Hoang, and J. S. Shin, "Protein Pattern Changes in Tomato under in vitro Salt Stress *," vol. 54, no. 4, pp. 526–533, 2007.

[16] P. Carillo, M. Grazia, G. Pontecorvo, P. Woodrow, and A.

Fuggi, “Salinity Stress and Salt Tolerance,” Abiotic Stress Plants - Mech. Adapt., no. September, 2012.
[17] M. Trovato, R. Mattioli, and P. Costantino, “Multiple roles of proline in plant stress tolerance and development,” Rend. Lincei, vol. 19, no. 4, pp. 325–346, 2008.
[18] S. I. Tanaka, S. Nakamura, N. Mochizuki, and A. Nagatani, “Phytochrome in cotyledons regulates the expression of genes in the hypocotyl through auxin-dependent and -independent pathways,” Plant Cell Physiol., vol. 43, no. 10, pp. 1171–1181, 2002.
[19] A. Bybordi, S. J. Tabatabaei, and A. Ahmedov, “Effect of salinity on the growth and peroxidaseand IAA oxidase activities in canola,” J. Food, Agric. Environ., vol. 8, no. 1, pp. 109–112, 2010.
[20] FN Dalton, A Maggio and G Piccinni ,“Effect of root temperature on plant response functions for tomato: comparison of static and dynamic salinity stress indices.” in Plant Soil, 192, pp:307-319, 1997.
[21] R Romero-Aranda, T Soria and JJ Cuartero , “Greenhouse mist improves yield of tomato plants grown under saline conditions.” in J Am Soc Hortic Sci, 127, pp:644-648, 2002.
[22] A Maggio, S De Pascale , G Angelino, C Ruggiero and G Barbieri , “Physiological responses of tomato to saline irrigation in long-term salinized soils.” in Eur J Agron, 21, pp:149-159, 2004.