EFFECT OF SALT STRESS ON CULTIVARS OF *LYCOPERSICON ESCULENTUM* MILL.: A COMPARATIVE BIOCHEMICAL APPROACH

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ABSTRACT

Knowledge of salt tolerance in vegetable plants is necessary to increase productivity and profitability of crops irrigated with saline wastewater. Tomato is moderately sensitive or moderately tolerant to salinity depending on cultivar or growth stage. Large genetic variation of tolerance to salt level exists among tomato genotypes. However, salt tolerance breeding programs have been restricted by the complexity of the trait, insufficient genetic and physiological knowledge of tolerance-related traits, and lack of efficient selection domain. Study on the physiological responses of tomato plants to salt stress could give novel insight into the planting and modifying of tomato cultivars. Studies were conducted to investigate the effect of short-term salinity stress on some physiological and biochemical alterations in two tomato cultivars during vegetative stage. Tomato cultivars grown in green house were treated with 2% NaCl for a short period in hydroponic conditions. Followed by physiological (root and shoot length, Wet and dry weight) and biochemical parameters (Chlorophyll and carotenoid content, proline, catalase, peroxidase etc.) were analyzed periodically in tomato leaves. The plantlets were widely affected in turn shunted plant growth was expressed in tested cultivars under salt stress. The genotypes exhibited different responses in terms of different osmo-protectant, antioxidant and pigment level. Plant photosynthetic and other pigments were slowly decreased, further elevated level of protein, phenol, carbohydrate, proline, ascorbate, catalase, polyphenol oxidase, lower H_2O_2 and hormones were observed in cultivar 1 associated to cultivar 2. Cultivar 1 could rapidly evolve physiological and antioxidant mechanisms to adapt the salt and manage the oxidative stress. It remained concluded that, tomato cultivar 1 partaking added salt tolerance capacity might have moderates the possibility to demise of the plantlets in the field condition underneath saline soil / saline water irrigation.

Key words: NaCl, Tomato, Salt stress, Physiological and Biochemical analysis.

1. INTRODUCTION

Tomato is an edible, most frequently consumed fruit belongs to *Solanum lycopersicum* in many countries, being the main source of several phytonutrients and providing important nutritional value to the human diet (Willcox *et al.*, 2003). During their lifespan, plants are frequently exposed to various stress factors. Based on their origins, stress factors are classified into 2 groups: abiotic and biotic stress factors (Mahajan and Tuteja, 2005).

Soil salinity is one of the key abiotic stress that largely depending on soil inherent mineral and chemical composition, and adversely disturb crop productivity. About 40% of agricultural lands worldwide were under threat of salinity. Today, almost 1 million hectares, corresponding to 7% of the earth's surface area, are under threat of salinity (Metternicht and Zinck., 2003) and such an area may influence up to 50% by the year 2050 unless measures are taken (Yaycili and Alikamanoglu, 2012). Amending saline condition in field would be expensive and temporary, while selection and breeding of salt tolerance can be a wise solution to minimize

salinity effects as well as improve production efficiency. Thus, identification and breeding tolerant cultivars under saline conditions is needed.

Knowledge of salt tolerance in vegetable plants is necessary to increase productivity and profitability of crops irrigated with saline waters. According to USDA report, out of all vegetables, tomato is moderately sensitive or moderately tolerant to salinity depending on cultivar or growth stage (Estan *et al.*, 2005). Study on the physiological responses of tomato plants under salt stress could give novel insight into the planting and modifying of tomato cultivars. The purpose of this study was the determination of mechanism contributed towards the salt tolerance in 2 different tomato cultivars during the vegetative stages. Such comparisons help to evaluate their relative performance and their salt tolerance.

2. MATERIALS AND METHODS

2.1 Genetic materials

Two tomato (*Lycopersicon esculentum* Mill.) genotypes collected from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, and India were used. The investigations were conducted under greenhouse bench of Nehru Arts and Science College, Coimbatore.

2.1.1 Plant growth conditions

The collected seedings were surface sterilized, sown in plastic pots containing soil mix (soil: vermiculite: organic fertilizer, 3:2:1, w/w/w) under greenhouse conditions (16 / 8 - h photoperiod; 26°C). The developed seedling was used for stress induction.

2.2 Stress induction in tomato plantlets

The tomato seeds were maintained in greenhouse conditions germinated after 8 days of planting. The plantlets were divided into two groups of ten plants each; one group served as control; the second was induced with salt stress.

For salt stress, test plants were irrigated with 2 % (w/w) of NaCl whereas control plants were received normal watering throughout the experiment. The uppermost fully expanded leaf samples were collected at 0, 3, 6, 9, 12, 15, and 18 days after stress (DOS) from control and salt stressed plants. The collected leaf tissues were quick-frozen and stored at -80ºC until further use.

2.2.1 Estimation of stress mediated physiological and biochemical changes

Physiological and biochemical response in plants under salinity stress was studied using standard procedures with slight modifications wherever applicable.

2.2.2 Physiological analysis

A total of around 40 uniform plants were used in one experiment with six replicates. The treatment was applied only once as a root drench during the experiment. The plants after

treatment were allowed to grow for the next two weeks. Plants were harvested at 2-3 days of interval and No. of leaves, Leaf area (cm^2), Root & shoot length (cm) and Root area (cm^2) were measured manually. The fresh weight (g) and dry weight (g) was also recorded. Control plants without salt stress also maintained along with the experimental setup. The experiment was repeated thrice during the year 2016-2017.

2.2.3 Biochemical analysis

Biochemical response like Total chlorophyll content and carotenoid content (Arnon, 1949), Total lycopene (Zakaria *et al.*,1979), Total flavonoids (Cameron, 1943), total phenol, Total carbohydrate (Yemm and Willies, 1954), Lipid peroxidation (MDA content) (Heath and Packer, 1968), Proline content (Bates *et al.*, 1973), Total Soluble Protein (Bradford, 1976), Total Ascorbate (Oser, 1979), Total H_2O_2 (Sagisaka, 1976), Catalase activity (Chance and Maehly, 1955), Peroxidase activity (Kar and Mishra, 1976), polypherol oxidase (Esterbauer *et al.*, (1977), Phytohormones analysis like GA3 estimation Berrios *et al.*, (2004) with minor modifications, IAA estimation (Ehmann, 1977) with reference to salinity stress was studied and the results were interpreted.

3.RESULTS

Salt tolerance in 2 different tomato cultivars in terms of in physiological and biochemical response during the vegetative were tested by adding 2% NaCl solution.

3.1 Physiological analysis

The dry weight and wet weight of leaves was decreased in 9^{th} day for cultivar 1, whereas for the cultivar 2 it was decreased in 12^{th} day. In connection with this, root and shoot length was increased till 6^{th} day followed by decrease in shoot and root length were observed in both cultivar 1 and cultivar 2 (Table 1).

3.2 Biochemical analysis

Biochemical responses to salinity stress was studied using standard procedures and the responses were recorded (Table 2 & 3).

Table 1. Effect of salinity stress on physiology of two different tomato cultivars

Genotype	Treatment	Wet wt. (mg/plant)	Dry wt. (mg/plant)	Root length (mg/plant)	Shoot length (mg/plant)
Cultivar 1	Control	39.13	10.53	4.93	3.32
	3rd DOS	32.3	6.8	5.43	3.39
	6th DOS	28.56	6.7	4.96	3.26
	9th DOS	17.5	5.2	3.91	2.87
	12th DOS	11.2	3.6	2.87	1.23
	15th DOS	4.5	1.2	0.98	0.43
	18th DOS	0.98	0.05	0.11	0.06
Cultivar 2	Control	44.67	15.06	5.16	2.98
	3rd DOS	35.4	8.16	5.03	3.04
	6th DOS	31.63	7.23	4.63	3.13
	9th DOS	24.04	6.3	3.04	3.01
	12th DOS	14.4	4.87	2.11	2.97
	15th DOS	9.25	2.52	1.78	1.67
	18th DOS	1.98	0.75	0.09	0.02

3.2.1 Pigments examination

The level of chlorophyll was decreased as 1.55mg/l in 18th day in cultivar 1 were as in the cultivar 2 chlorophyll level of 1.73 mg/l was observed in 12th day. Carotenoid content in leaves was decreased in 15th day for cultivar 1 which showed 0.098mg/g were as the cultivar 2 on 18th day showed as 0.032mg/g. Flavonoids content in leaves was increased till 9th day for cultivar 1 as 0.923mg/l then gradual decrease was observed. In cultivar 2, it was observed till 6th day (0.884mg/l) then decreased. Lycopene content in leaves was increased in 12th day for cultivar 1 as 0.539mg/g were as the cultivar 2 the lycopene content was decreased in 6th day as 0.513mg/g.

3.2.2 Total phenol and Carbohydrate content

The level of phenol content in leaves was decreased as 0.056mg/l on 12th day for cultivar 1 and cultivar 2 showed it on 15th day as 0.051mg/l. Carbohydrate content in leaves was stated decreasing in 6th day for cultivar 1 & 2 as 1.84 mg/l.

3.2.3 Lipid peroxidation and Proline content

Lipid peroxidation content in leaves were decreased in 9th day as 1.002mg/l for cultivar 1 and cultivar 2 showed decrease from 15th day only (1.001mg/l). Increase in proline content (0.912mg/l) was observed on 9th day for cultivar 1 and for the cultivar 2 it was observed as 0.926mg/l.

3.2.4 Protein, Ascorbate and H_2O_2content

For cultivar 1 protein content was decreased as 0.05 mg/l on 15th day, were as the cultivar 2 showed decreased protein content on 12th day as 0.03 mg/l. Ascorbate content in leaves was increased in15th day for cultivar 1 as 0.05mg/l were as the cultivar 2 it increased in 12th day as 0.03 mg/l. The level of H_2O_2 content in leaves was increased in 6th day for cultivar 1 as 1.165 mg/l were as the cultivar 2, increased in 9th day as 1.297 mg/l.

3.2.5 Catalase, peroxidase and polypherol oxidase content

Catalase content in leaves was increased in 9th day for cultivar 1 as 1.012 mg/l and for cultivar 2 as 1.074 mg/l. Peroxidase content was increased in 15th day for cultivar 1 as 2.207 mg/l, in contrast cultivar 2 showed increase on 18th day as 2.214 mg/l. The level of polypherol oxidase content in leaves was increased in 9th day for cultivar 1 as 0.985 mg/l were as the cultivar 2 as increased in 6th day as 0.974 mg/l.

3.2.6 Hormone (GA_3 & IAA) content

The hormone (GA_3) content in leaves was decreased in 15th day for cultivar 1 as 0.057 mg/l were as the cultivar 2 was decreased in 12th day as 0.044 mg/l. Likewise IAA content in leaves was decreased in 15th day of cultivar 1 as 0.072 mg/l were as the cultivar 2 showed decreased in 6th day as 0.08 7mg/l .

4. DISCUSSION

4.1 Physiological analysis of tomato plantlets under salt stress

Two tomato (*Lycopersicon esculentum* Mill.) genotypes were collected and the seedings were kept under salt stress (2%). The plant roots are the first site within the plant to be impacted by osmotic changes in the soil environment. Soil water deficit or fluctuations in the ionic profile of the root zone are important factors controlling water movement in the plant root. Thus, monitoring the inhibition of root growth is an important screening criterion for tolerance to salinity (Borsani *et al.*, 2001).

In the present study, physiological analysis showed dry weight, wet weight, root length and shoot length were decreased in cultivar 2 than cultivar 1 while inducing salt stress. This increased shoot and root lengths as compared to high salt stress may be due to enhanced cell

wall extensibility of the primed seeds. Turhan *et al.*, (2016) reported that the dry weight of the 12 tested tomato crosses were found to be lowered with the rise of salinity level on comparing with control. Similarly, Jamil *et al.*, (2006) reported that both root length and root surface area per plant were decreased significantly under higher salinity conditions. Compared to the lowest salt level and the highest salt level decreased the root length by 49%, 55%, and 62% at the 0.5,1, and 2 mMP levels of phosphorus, respectively. Similar observations were obtained by Hajer *et al.*, (2006), Maggio *et al.*, (2006) and Li and Stanghellini, (2001).

Table 2. Effect of salinity stress on biochemical permeameters of tomato cultivar 1

Tests	Control	3rd DOS	6th DOS	9th DOS	12th DOS	15th DOS	18th DOS
Chlorophyll	39.62± 0.79	40.19± 0.99	14.52± 1.20	9.38± 0.64	2.38± 0.32	2.15± 0.29	1.55± 0.57
Carotenoid	1.07±0.06	0.87±0.06	0.65±0.11	0.60±0.06	0.22±0.10	0.09±0.00	0.02±0.01
Lycopene	0.23±0.001	0.38±0.001	0.41±0.001	0.44±0.001	0.53±0.001	0.61±0.001	0.64±0.001
Flavonoids	0.36±0.001	0.57±0.001	0.86±0.001	0.92±0.001	0.74±0.001	0.58±0.001	0.42±0.001
Total phenol	0.52±0.001	0.39±0.001	0.21±0.001	0.10±0.002	0.05±0.001	0.02±0.001	0.14±0.001
Carbohydrate	2.20±0.01	2.19±0.01	1.84±0.10	1.75±0.09	1.65±0.09	1.40±0.11	1.26±0.06
Lipid peroxidation	1.99±0.003	1.54±0.002	1.29±0.002	1.00±0.002	0.85±0.001	0.61±0.01	0.48±0.001
Proline	0.10±0.02	0.28±0.09	0.85±0.07	0.94±0.07	1.24±0.11	1.65±0.09	1.96±0.06
Protein	0.13±.01	0.26±.01	0.17±0.01	0.11±0.01	0.08±0.01	0.05±0.01	0.009±0.00
Ascorbate	0.01±0.00	0.01±0.00	0.02±0.00	0.04±0.00	0.04±0.00	0.06±0.00	0.08±0.01

H_2O_2	0.67±0.01	0.89±01	1.16±0.01	1.30±0.01	1.85±0.00	1.92±0.00	2.31±0.02
Catalase	0.34± 0.08	0.82± 0.07	0.87± 0.11	1.32± 0.58	1.12± 0.11	2.12± 0.19	2.37± 0.27
Peroxidase	0.12±0.01	0.45±0.01	0.94±0.00	1.42±0.01	1.83±0.01	2.20±0.00	2.33±0.01
Polyphenol oxidase	0.41±0.02	0.65±0.01	0.78±0.02	0.98±0.01	1.02±0.02	1.27±0.01	1.46±0.01
GA3	0.23±0.03	0.11±0.00	0.08±0.01	0.06±0.01	0.05±0.01	0.03±0.01	0.01±0.00
IAA	0.18±0.00	0.16±0.01	0.14±0.01	0.11±0.01	0.09±0.00	0.07±0.01	0.04±0.01

Values are Mean ± SD of Triplicates

Table 3. Effect of salinity stress on biochemical permeameters of tomato cultivar 2

Tests	**Control**	**3rd DOS**	**6th DOS**	**9th DOS**	**12th DOS**	**15th DOS**	**18th DOS**
Chlorophyll	22.42±1.17	19.64±0.74	11.62±1.16	8.68±0.68	1.73±0.16	1.23±0.27	1.00±0.34
Carotenoid	1.15±0.01	0.86±0.01	0.65±0.06	0.55±0.06	0.38±0.10	0.11±0.01	0.03±0.00
Lycopene	0.41±0.001	0.46±0.001	0.51±0.001	0.63±0.001	0.71±0.001	0.77±0.001	0.84±0.001
Flavonoids	0.48±0.0001	0.62±0.001	0.88±0.001	0.63±0.001	0.53±0.00	0.44±0.001	0.39±0.001
Total phenol	0.31±0.001	0.29±0.001	0.17±0.001	0.92±0.001	0.06±0.001	0.05±0.001	0.42±0.001
Carbohydrate	2.18±0.05	2.21±0.21	1.84±0.08	1.74±0.10	1.64±0.14	1.35±0.09	0.84±0.12
Lipid peroxidation	2.02±0.001	1.83±0.002	1.72±0.002	1.50±0.001	1.28±0.001	1.00±0.002	0.83±0.001
Proline	0.18±0.14	0.84±0.07	0.89±0.07	0.92±0.07	1.44±0.22	1.44±0.10	1.74±0.11
Protein	0.09±0.01	0.16±0.01	0.12±0.01	0.08±0.01	0.03±0.01	0.02±0.01	0.005±0.00
Ascorbate	0.00± 0.00	0.02± 0.01	0.03± 0.01	0.04± 0.01	0.05± 0.01	0.07± 0.01	0.09± 0.01
H_2O_2	0.56±0.01	0.79±0.01	1.00±0.02	1.29±0.01	1.64±0.01	1.80±0.00	2.27±0.00
Catalase	0.35± 0.12	0.83± 0.04	0.89± 0.03	1.03± 0.08	1.17± 0.01	2.12± 0.10	2.36± 0.09
Peroxidase	0.09±0.01	0.25±0.00	0.87±0.01	0.99±0.01	1.52±0.00	2.02±0.06	2.21±0.00
Polyphenol oxidase	0.70±0.007	0.86±0.006	0.96±0.007	1.04±0.008	1.19±0.01	1.34±0.01	1.48±0.009
GA3	0.22± 0.04	0.20± 0.01	0.09± 0.01	0.07± 0.01	0.06± 0.01	0.04± 0.01	0.02± 0.00
IAA	0.17±0.01	0.15±0.00	0.13±0.01	0.10±0.00	0.08±0.00	0.06±0.00	0.02±0.00

Values are Mean ± SD of Triplicates

4.2 Biochemical analysis of tomato plant under salt stress

According to biochemical analysis, the two different cultivars differ greatly in their response to salinity. In the present investigation, NaCl stress in tomato plants increased the level of proline, lycopene, ascorbate, polypherol oxidase, H_2O_2 and peroxidase content whereas chlorophyll, flavonoid, protein, hormones and carbohydrate contents were decreased.

Chlorophyll content becomes a first indication of responses in different plants under salinity stress. The cultivar 1 showed enhanced chlorophyll content whereas in cultivars 2, the inhibition was clearly visible. The result may be due to better adaptation and resistance to salinity stress of cultivar 1. Like our evidence, Dogan *et al.,* (2010) reported that chlorophyll concentration was lesser in salt-sensitive cultivars than in salt-resistant cultivars of tomato. Also, Tantawy *et al.,* (2009) observed the decrease in total chlorophyll content in tomato with the increasing level of salinity.

Carotenoids are the pigments in plants which protect plants from oxidative stress. Carotenoids present in photosynthetic organisms absorb the light and transfer to chlorophyll for photosynthesis. In addition, carotenoids also help to protect the plants from damage caused by light (Taiz and Zeiger, 2010). Therefore, higher carotenoids in cultivar 1 must be an attributor in this regard. Here we could indeed demonstrate that the level of β-carotene as unaffected by salinity stress in cultivar 2 may be because of such short-term stress whereas in cultivar 1 enhancement was upto 1.5-fold over its control may be due to its better tolerance to salinity. Carotenoids has been reported one of the non-enzymatic antioxidants which play an important role in the protection against oxidative stress was reported by Kojo, (2004).

Flavonoids are largely responsible for coloration in flowers and fruits of higher plants (Harborne, 2000) also it protects cells from excessive UV-B radiation (Taiz and Zeiger, 2010). In our experiment higher accumulation of flavonoid in cultivar 1 is certainly pointing towards a defence mechanism. In other reports where researchers found that, low molecular weight antioxidants like polyphenols (Sgherri *et al.*, 2004), flavonoids (Hernandez *et al.*, 2004) and carotenoids (Strzalka *et al.*, 2003) can effectively scavenge harmful radicals and stabilize lipid oxidation.

Many plants accumulate high levels of proline in response to osmotic stress, and its play an adaptive role during osmotic stress. Under salt stress most plant species exhibited a remarkable increase in their proline content (Dasgan *et al.,* 2009; Patel and Pandey, 2008). In our experiments a similar observation in respect to proline content was found to be higher when plants were exposed to salt stress. Higher level of proline content may be due to expression of

genes encoding key enzymes of proline synthesis Pyrroline-5-carboxylate(P5C) and low activity of the oxidising enzymes (proline dehydrogenase) which is controlled by osmotic and salinity stress. Tabatabaei and Naghibalghora., (2013), reported that ascorbate increased seed proline in normal situation without any stress while it decreased seed proline in stress situation. Many reports are in support to our findings like when exposed to high salt content in the soil, many plants accumulated high amounts of proline (Claussen, 2005, Mansour and Salama, 2004, Mansour, 2000).

Like proline, in recent years, the role of sugars in saline stress was controversial. In the present work, the maximum concentration of carbohydrates was found in the most salt-resistant cultivar, whereas the lowest was seen as the salt-sensitive tomato cultivar. While soluble carbohydrates accumulation in plants has widely been documented against salinity response (Ahamed *et al.*, 2010; Ashraf and Harris, 2004 and Murakeozy *et al.*, 2003). Likewise, NaCl mediated changes in soluble proteins were observed in two cultivars. The rise of soluble protein at low salinity and decreases with high salinity in mulberry cultivars has already been observed by Agastian *et al.*, (2000). Plants under stress may be expected to have a powerful protein turnover machinery to destroy stress and ecologically regulated proteins (Abdel *et al.*, 2003).

Malondialdehyde (MDA), a lipid peroxidation product, has been regarded as an oxidative damage indicator (Shalata and Neumann, 2001). The controlled lipid peroxidation in cultivar 1 must have given a better protection against oxidative damage under salt stress. There are reports of enhancement in MDA content initially with the increase in salt stress in the salt sensitive cultivar as compared to tolerant cultivar of rice was reported by Roychoudhury *et al.*, (2011) and in roots of rice varieties was reported by Khan and Panda, (2008).

Younis *et al.*, (2010), reported that the growth reduction caused by salinity stress is due to inhibited apical growth because of endogenous hormonal imbalance. In addition, a secondary aspect of salinity stress in plants is the stress-induced production of ROS lead to the progressive oxidative damage and ultimately cell death and growth suppression (Manchanda and Garg, 2008).

Salt discomfort induces a stomatal contraction, reducing the ratio of carbon dioxide and oxygen in plant cells. The excess oxygen leads to the formation of reactive oxygen species (ROS), it reaches dangerous levels when a plant is under abiotic stress (Xiong and Zhu, 2002, Roxas *et al.*, 2000). In our experiment, the H_2O_2 generation was found to be elevated by about 80% in cultivar 2 but no marked difference in cultivar 1. It may be interpreted that cultivars 1 have better adaptive mechanism in scavenging H_2O_2. H_2O_2 accumulation and lipid

peroxidation in sensitive pea and rice cultivars were identified as higher under stressful conditions (Lee *et al.*, 2008, Hernandez and Almansa, 2002).

Catalase and peroxidase are the main enzymes involved in the detoxification of the deleterious oxygen species (Mittova *et al.*, 2002). Expression of the ROS genes in plants under abiotic stress may be predicted to be upregulated (Zhu *et al.*, 2005). Moreover, in reaction to salinity (ROS), the capacity of certain plants to increase the generation of antioxidant compounds and enzymes was linked to the salt tolerance (Shalata and Neumann, 2001). In the present study, antioxidant enzyme activities changed significantly in response to the salinity stress. catalase and peroxidase activities in the leaves of the tolerant genotype increased with increasing salinity over the control plants, and then decrease slightly at higher salinity level. Similarly, catalase inhibition by salt stress was also observed in Rye, Vigna and rice (Singha and Choudhuri, 1990; Hertwig *et al.*, 1992).

5. CONCLUSION

Data presented here suggested that even in very short term of salt stress brought the changes at the biochemical level that can help in the identification of tomato genotypes of salinity tolerance. Among the two cultivars studied, cultivar 1 has more salt tolerant capability than cultivar 2. Based on conventional plant physiology approaches, plantation of cultivar 1 decreases the possibility to death of the plantlets in the field condition. Further, genetics approaches to study the plant responses to abiotic stresses may serve as a confirmation criterion for salt tolerance genotype in agricultural crop improvement.

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