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THE EFFECT OF SALINITY ON GROWTH, HORMONES AND MINERAL ELEMENTS IN LEAF AND FRUIT OF TOMATO CULTIVAR PKM1

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ABSTRACT

Tomato cultivar PKM 1 were subjected to 25, 50, 100, 150 and 200 mM NaCl stress and response of tomato plant to salt stress were determined by assessing the variability of different biochemical parameters. In this present study endogenous content of growth hormones IAA and ABA in leaves, proline and mineral (Na+ and K+) content in leaves and mature fruits were estimated. Leaf area and dry matter content of tomato fruits under salt stress were determined to study the effect of salinity on photosynthetic yield. Results showed that leaf area and dry matter content of tomato fruits decreased with application of elevated salt stress, however endogenous content of IAA, ABA and proline was found to be increasing with increase in salt treatment. Application of NaCl caused increase in Na+ content, while K+ content and K+/Na+ ratio decreased with increase in salt stress. Another striking point is that increase in proline and Na+ content was more in leaves than fruits, which suggests that leaves are more sensitive than fruits.
Keyword: Salt stress, IAA, ABA, Proline, Na+, dry matter

INTRODUCTION

Salt stress is one of the major abiotic stress factors that affect almost every aspect of physiology and biochemistry of a plant, resulting in a reduction in its yield (Foolad, 2004). Thus it is a serious threat to agricultural productivity especially in arid and semi-arid regions (Parvaiz *et al.*, 2008). Salt stress causes hyperosmotic stress and ion disequilibrium, thereby disabling the vital cellular functions of a plant. Reduced availability of water, increased respiration rate, altered mineral distribution, membrane instability, failure in the maintenance of turgour pressure are some of the events that prevails during this stress episode. Plants try to withstand these stresses either by tolerating it or by adopting a dormant stage (Cuartero *et al.*, 2006). Salt tolerance is a complex trait which involves numerous genes and various physiological and biochemical mechanisms (Cuartero *et al.*, 2006). Salt tolerance effectors and regulatory components gain importance at this juncture. Despite recent advances in molecular marker technology, QTL mapping, marker assisted selection, genetic transformation, a successful salt tolerant tomato derivative from agronomic point of view hasn't come yet. Tomato plant is considered as a model crop to examine the potentiality of marker assisted selection and genetic transformation as its genes are well studied than any other dicotyledons crop and it can be transformed through various methods (Cuartero *et al.*, 2006). Improvement for salinity tolerance would be of significant value for a moderately sensitive crop like tomato when it is grown on lands with salinity problems. Extensive research is being carried out to produce tomato plants with improved salt tolerance. Development of transgenic tomato plants overexpressing *AtNHX1*, a single-gene controlling vacuolar Na+/H+ antiport protein, introduced from *Arabidopsis thaliana* is an example for the transgenic approach towards salt tolerance (Zhang and Blumwald, 2001). But salinity varies according to time and space. Hence the experimental design must allow the study of genotype X environmental interaction (Cuartero *et al.*, 2006; Foolad, 2004). The tomato cultivar PKM1 is commonly consumed all over South India. This study on various parameters such as the levels of phytohormones, osmolytes, ion contents and other morphological characters such as plant height, no. of fruits /plant, leaf area, chlorophyll content, of this tomato variety will provide new insights in exploring the genetic determinants of salt tolerance and thus increase the yield.

MATERIALS AND METHODS

Plant material and salt treatment: Tomato cultivar PKM 1 was used as plant material in this study. PKM 1 variety seeds were purchased from local dealers and grown at VIT University, Vellore. Six sets of plant (three biological replicates each) were treated with six different concentration of NaCl such as 25 mM, 50 mM, 100 mM, 150 mM and 200 mM respectively. Soils were watered with water containing respective concentration of NaCl as stated above immediately after sowing and watering was carried out for 90 days, after which the samples were collected for further experiments.

Extraction and quantification of plant hormones Abscisic acid (ABA) and Indole acetic acid (IAA): Leaves from both salt treated and control plants were collected for extraction and quantification of ABA and

IAA after 90 days of treatment. Extraction and quantification of ABA and IAA was done according to protocol given by Horemans *et al.* (1984). ABA and IAA were quantified using C18 reverse phase High Performance Liquid Chromatography (HPLC). Samples were isocratically eluted at 2ml per minute with methanol: 2% acetic acid: water (40:40:20) as a mobile phase. Detection was done with an absorbance monitor operating at 264nm.

Estimation of Proline, Na+ and K+ content: Fully expanded leaves from salt treated and control plants were collected at 90 days after salt application and mature tomato fruits were also collected for estimation of proline, Na+ and K+ content. Proline content was estimated spectrophotometrically by acid ninhydrin procedure (Bates *et al.* 1973). Leaves and fruits samples were digested with nitric acid: perchloric acid (4:1) mixture. Na+ and K+ content were estimated by flame photometry (Horneck and Hanson, 1998) and K+/Na+ ratio was calculated.

Determination of leaf area and dry matter percentage: Leaf area was calculated by graph shadowing method. Leaves were laid on a graph paper (1square = 1 cm2) and their outline was traced. Number of square centimeter was counted (partial square if it is at least half covered by the leaf, partial squares which were not half covered were ignored). This method gives an approximate leaf area. Dry matter percentage of tomato fruits were estimated at 105˚C for 16 hrs in a hot air oven.

Statistical analysis: The Graphpad Instat 3 version statistical software were used to calculate the means, standard errors and standard deviations. Statistical analysis one-way ANOVA was applied to the data obtained to determine the differences in the parameters (levels of abscisic acid, indole acetic acid, proline, sodium ion, potassium ion, leaf area, plant height, dry matter percentage) under various concentrations of NaCl in a randomized experimental design.

RESULTS AND DISCUSSION

Effect of salt stress on growth parameters: Salt tolerance is developmentally regulated in a plant life cycle and it is stage specific. Research in this area has been mostly confined to early stages of plant life usually by providing salt stress for short duration of time as shock treatments. But salt tolerance at vegetative stage and reproductive stage has to be focused more than germination stage because most tomato crops are established as transplanting seedling than direct seeding (Foolad, 2004). In spite of that a positive correlation is observed between tomato yield and plant size during these stages which states the importance of the study (Bolarin *et al.*, 1993). Few studies have been carried out in the vegetative and reproductive stage of tomato plants. Thus in a view to understand the physiological and biochemical mechanisms underlying the salt tolerance at reproductive stage, samples were selected from plants exposed to various concentrations of NaCl for 90 days.

Salt stress affects the plant growth and development thereby affecting the yield quantity and quality (Foolad, 2004; Maggio *et al.*, 2002; Cuartero *et al.*, 2006; Sattar *et al.*, 2010). A successful salt tolerant cultivar should exhibit salt tolerance without compromising its yield potential. Therefore some of the yield characters such as plant height and number of fruits per tomato plant have to be taken into consideration in this study. The response of tomato cultivar PKM1 to increasing concentrations of NaCl as 25 mM, 50 mM, 100 mM, 150 mM and 200 mM in which a significant decrease in plant height and number of fruits per plant was seen as the salt concentration increased (Table 1). The previous studies denotes that a reduced growth at low concentrations of salt is caused by less availability of nutrients require for its growth. As salt concentration increases besides nutrient imbalance, hyperomostic stress and ion disequilibrium plays a pivotal role in disturbing the cellular functions of plant (Foolad, 2004).

Dry matter content of mature tomato fruits was found to be decreased with application of elevated salt treatment (Table 1). The dry matter content of tomato fruit ranged from 10.23% to 2.89%. The present results are in agreement with those reported by Majkowska *et al.* (2008). As reported by Cuartero and Fernandez-Munoz (1999), dry matter is crucial quality parameters for both fresh market and processing tomatoes. To improve the quality of processed products, the high dry matter content is desired, the average dry matter content of fresh mature tomato fruits must be at least 5% for use in fresh market and processing tomatoes (DePascale *et al.*, 2001). In this present study dry matter of mature tomato fruit was significantly decreased to 2.78%, which indicates that tomato fruits grown under saline condition contain low dry matter and these fruits are not good for both fresh market and processed products.

In developing salt tolerant tomato cultivars, heritability of the selected trait has to be considered along with its physiological and metabolic importance. Leaf area showed the highest heritability as compared to shoot dry weight, measures of ion contents and water relations (Cuartero *et al.*, 2006). Present study showed significant decrease in leaf area of tomato leaves with application elevated salt treatment (Table 1). Under saline condition as soon as new cell starts its elongation process, the excess of Na+, Cl- and other ions modifies the metabolic activities of cell wall, which causes deposition of several materials on cell wall and limits the cell wall elasticity (Yasar *et al.*, 2006). Cell walls become rigid and turgor pressure efficiency in cell enlargement is decreased with application of elevated salt treatment. The other anticipated cause of reduction in leaf area and dry matter content could be the reduced development and differentiation of tissues, shrinkage of the cell contents, unbalanced nutrition, damage of membrane and disturbed avoidance mechanism (Akram *et al.* 2007).

Effect of salt stress on sodium and potassium ion concentration: Salinity not only affects yield potential of a plant but almost every aspect of physiology and biochemistry of a plant (Parvaiz *et al.*, 2008; Zhu, 2002). The physiological characters that display a genetic variability in the available germplasm has to be selected for the analysis. According to Cuartero *et al.* (2006) four physiological characters showed a variability in tomato: Na+ transport to the shoot from the external root nutrient solution, the relation between leaf Na+ and leaf area reduction, the capacity to accumulate Na+ in old leaves while young leaves maintain a low Na+ and leaf K+/Na+ ratio.

Chemical analysis of leaf and mature fruits showed a significant elevation in the levels of sodium ion concentration while K+ and K+/Na+ decreased with application of higher concentrations of NaCl (Table 2 and 3). The rates of increase in Na+ content were higher in leaves than in fruits. The distribution of Na+ varies among the organs of the plant. Due to this accumulation of Na+ in different parts of plant differs (Hasegawa *et al.*, 2000). Similar studies were carried out and same outcomes were found by some other authors (Loukehaich *et al.*, 2011; Hu and Schmidhalter, 1997; Sagi *et al.*, 1997; Bagcı *et al.*, 2003; Beck *et al.*, 2004; Netondo *et al.*, 2004; Akram *et al.*, 2007).

Potassium content was found to be decreasing with increase in salt stress (Table 2 and 3). These outcomes suggest that there was a competition between Na+ and K+ regarding their uptake. Similar findings were reported with soybean cultivars (Essa, 2001; Li *et al.*, 2006), green bean cultivars (Yasar *et al.*, 2006) and canola cultivars (Bandeh-Hagh *et al.*, 2008).

Tomato plants under salt stress exhibited a decrease of K+/Na+ ratio in both leaves and mature fruits. Our current findings are in agreement with other reports proposing that salt stress reduces the K+/Na+ ratio of wheat (Hu *et al.*, 2006), melon (Kusvuran *et al.*, 2007), green bean (Yasar *et al.*, 2006) and legume (Amador *et al.*, 2007). On the basis of previous studies and current findings it can be postulated that K+/Na+ ratio shows positive relationship with salt tolerance and it might be a valid selection criteria for evaluating the salt tolerance of different crop species (Essa, 2001; Kusvuran *et al.*, 2007). The Na+ accumulation in plants cause many deleterious effects such as necrosis of leaves and reduced shoot and root growth (Munns, 2002). The accumulation of Na+ interferes with the K+ selective ion channels in the root plasma membrane and thus reduce the availability of many nutrients (Tester and Davenport, 2003) .The reduced intake of K+ ions hinders protein synthesis as it plays a major role in binding tRNA to ribosomes (Blaha *et al.*, 2000). It's reported that Na+ transport from root to shoot is unidirectional and the resultant build up of Na+ in leaves causes osmotic damage (Flowers *et al.*, 1991).

Effect of salt stress on proline content in leaves and fruits: Plants under salinity stress synthesize some low molecular mass compatible solutes to increase the osmotica within the cell (Loukehaich *et al.*, 2011). These organic solutes help in maintaining the internal water potential below that of soil and thus maintain the turgor pressure. Proline, which is an aminoacid is one such organic solute that plays a major role in this osmotic adjustment. Biochemical analysis of leaves and mature fruits for proline accumulation indicated that proline accumulation increased with increase in salt stress (Table 4). A more pronounced increase was observed in leaves as compared to fruits which suggest that, leaves are under more stress than fruits. In the present study, a 10 fold increase in proline content was observed in leaves while a 3.6 fold increase of proline content was observed in mature fruits. In this case we observed positive relationship between proline accumulation and salt stress.

Increase in proline content during environmental stress is caused by activation of its biosynthesis and deactivation of its degradation. Capability to accumulate proline in response to environmental stress in highly variable between or within species (Lutts *et al.* 1996). Proline is synthesized in plants through two alternate pathways: L-ornithine and L-glutamate pathways (Parvaiz *et al.*, 2008). It acts as a signaling molecule initiating adaptation processes towards the stress episode (Maggio *et al.*, 2002). It provides membrane stability and alleviates the cell membrane disruption brought about by salinity stress (Mansour, 1998).

Effect of salt stress on phytohormones: There are many internal and external factors that affect the overall growth and development of plants. One such internal factor is plant hormone, which helps in transmitting signal between the cells and within the cells. Previous findings have conjectured that endogenous content of plant hormones such as abscisic acid, auxin, cytokinins, zeatin and gibberellins changes in response to salt stress (Javid *et al.*, 2011). In the present study, two important plant hormones ABA and IAA were estimated to explore the effect of salinity on plant hormones. Abscisic acid which is known as the hormone of stress is a major player in mediating adaptive responses of plants to stress (Javid *et al.*, 2011). It is produced in roots in response to decreased soil water potential and translocated to leaves where it binds to receptors at the surface of plasma membrane of stomatal guard cells. Present results showed that endogenous ABA and IAA content significantly increased with the application of elevated NaCl treatment (Table 5). This increase in endogenous ABA content hinders growth and development of tomato plant under salt stress and also causes closure of stomata affecting photosynthesis. Maximum content of ABA was observed at 200mM NaCl treatment.

Table 1: Leaf area, dry matter weight percentage, plant height and no. of fruits per plant

Treatment	Leaf Area (cm2)	Dry matter weight %	Plant height (cm)	No. of fruits per plant
Control	18.24 ± 0.31	9.943 ± 0.3252	161.88 ± 3.83	15
25 mM NaCl	16.45 ± 0.45	8.231 ± 0.130	149.72 ± 2.72	12
50 mM NaCl	15.38 ± 0.13	6.947 ± 0.0252	129.54 ± 2.63	10
100 mM NaCl	12.53 ± 0.51	5.231 ± 0.0529	116.55 ± 3.77	7
150 mM NaCl	11.28 ± 0.17	4.176 ± 0.1504	101.34 ± 2.55	6
200 mM NaCl	10.23 ± 0.29	2.786 ± 0.105	85.71 ± 4.02	4

Table 2: Levels of Na +, K+ and K+/Na+ in leaves

Treatment	Sodium ion (mM/g F.W)	Potassium ion (mM/g F.W)	K+/Na+ ratio
Control	4.63 ± 0.065	57.2 ± 0.011	12.35
25 mM NaCl	14.9 ± 0.0342	54.64 ± 0.022	3.66
50 mM NaCl	27.57 ± 0.049	49.04 ± 0.0426	1.77
100 mM NaCl	38.5 ± 0.096	44.43 ± 0.0286	1.151
150 mM NaCl	47.65 ± 0.079	41.05 ± 0.0234	0.861
200 mM NaCl	56.8 ± 0.0358	38.92 ± 0.0318	0.68

Table 3: Levels of Na +, K+ and K+/Na+ in fruits

Treatment	Sodium ion (mM/g F.W)	Potassium ion (mM/g F.W)	K+/Na+ ratio
Control	2.04 ± 0.507	39.58 ± 0.077	19.40
25 mM NaCl	6.38 ± 0.029	33.97 ± 0.049	5.32
50 mM NaCl	13.9 ± 0.119	27.64 ± 0.059	1.98
100 mM NaCl	29.9 ± 0.066	24.58± 0.033	0.82
150 mM NaCl	40.6± 0.083	21.69 ± 0.018	0.53
200 mM NaCl	55.69 ± 0.065	17.89± 0.063	0.32

Table 4: Levels of Proline in leaves and fruits

Treatment	Proline in leaves (µM/g F.W)	Proline in fruits(µM/g F.W)
Control	0.592 ± 0.028	0.513 ± 0.021
25 mM NaCl	0.865 ± 0.0055	0.743 ± 0.0414
50 mM NaCl	1.389 ± 0.0067	0.9367 ± 0.0328
100 mM NaCl	2.069 ± 0.036	1.23 ± 0.0426
150 mM NaCl	2.759 ± 0.011	1.453 ± 0.0569
200 mM NaCl	6.563 ± 0.029	1.8597 ± 0.0605

Table 5: Levels of Abscisic acid and Indole acetic acid in leaves

Treatment	Abscisic acid (mM/g F.W)	Indole acetic acid (mM/g F.W)
Control	0.484 ± 0.0025	0.587 ± 0.0025
25 mM NaCl	0.529 ± 0.0025	0.599 ± 0.0035
50 mM NaCl	1.0479 ± 0.0026	0.981 ± 0.0032
100 mM NaCl	2.065 ± 0.0036	2.203 ± 0.004
150 mM NaCl	8.17 ± 0.031	2.32 ± 0.0049
200 mM NaCl	24.7 ± 0.02	3.168 ± 0.0062

IAA controls almost all aspects of plant life, from seed germination to vegetative growth and flowering (Ritchie and Gilroy, 1998). In current study, endogenous bioactive IAA content significantly increased with application of elevated NaCl treatment (Table 5). It suggests that the growth and development of tomato plants under salt stress is supported by increased production of IAA. Our current finding are in agreement with the previous reports of (Wang *et al.*, 2001), who demonstrated that IAA generally increase in plants in response to elevated salt stress.

Conclusion and Future perspectives: The enhancement of salt tolerance in crops is highly challenging as it involves participation of multiple physiological and biochemical pathways. Thus a thorough knowledge on physiological mechanisms and genetic basis of the salt tolerance effectors and regulatory components is essential. The present work demonstrated that under saline condition *Lycopersicon esculentum* shows substantial amount of variation in physiological characteristics. Our major interest was to assess the extent of variability of different biochemical parameters in tomato cultivar PKM 1 under salt stress. This work clearly states that tomato plant grown under salt stress showed reduction in dry matter content and leaf area, which limits the biomass and photosynthetic yield of tomato plants. Endogenous phytohormones (IAA and ABA) content and proline content was found to be increasing to make the plant more salt tolerant. However, increase in Na+ content and decrease in K+ content disrupts ion homeostasis which affects the osmotic potential of cells and has negative effect on plant growth and development under saline condition. Further studies on genetics, molecular biology and comparative genomics is essential to discover novel salt tolerant genes. Functional analysis of those genes by gene silencing strategies helps in the development of successful salt tolerant crops. Conventional breeding, molecular marker technology, quantitative trait loci analysis, genetic transformation coupled with a practice of some culture techniques such as seed priming, seedling conditioning, increasing the relative humidity and grafting cultivars onto rootstocks help plants to withstand deleterious effects of salt in a better way.

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