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A STUDY ON CYTOTOXIC IONS SEQUESTRATION AND K^+/Na^+ LEVELS AS SALT TOLERANT INDICATORS IN TOMATO (*Solanum lycospersicum* L.)

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ABSTRACT

The study on ion homeostasis and cytotoxic ion sequestration in tomato (*Solanum lycospersicum* L.) was investigated. Salinity stress did not show a significant effect ($P>0.05$) on dry matter accumulation of shoots and roots of tomato. The accumulation of sodium ion (Na^+) in root of tomato increased in salt treated groups (50 mM, 75 mM and 100 mM) while in shoot of tomato, the Na^+ accumulation was highest (6 times higher than the control) in 75 mM treatment. Potassium (K^+) uptake was salt concentration dependent in both shoot and root and 50 mM treatment of each organ yielded highest K^+ content. The Na^+/K^+ levels in shoot and root increased with increasing concentrations but the magnitude of this level is lower in shoot than in root due to the high level of K^+ content in the shoot tissues. It can be concluded from these findings that Na^+ was compartmentalised both in shoot and root of tomato by membrane transporters and that low level of Na^+/K^+ level was a good indicator of salt tolerance property in the tomato genotype studied.

Keywords: Sequestration; *Solanum lycospersicum*; Salinity stress; ion homeostasis; membrane transporters, cytotoxic ions

INTRODUCTION

Salinity stress is a major constraint to food production because it limits crop yield and restrict the use of land previously uncultivated. Salinity affects plants through hyper osmotic effect, ion disequilibrium and oxidative stress (Hasegawa *et al.,* 2000; Zhu, 2001). The homeostasis of intra cellular ion concentrations is fundamental to the physiology of living cells. Proper regulation of ion flux is necessary for cell to keep the concentration of toxic ions low and to accumulate essential ions (Zhu, 2001). Plant cell employ primary active transport, mediated by H^+ -ATPases, and secondary transport, mediated by channels and co-transporters, to maintain characteristically high concentration of K^+ and low concentration of Na^+ in the cytosol. There are various ways by which plants can keep endogenous level of ions like Cl^- and Na^+ low. Reduced influx at the root cell plasma membrane, efflux from roots and retranslocation from the leaves to the roots are possible mechanisms (Bhandal and Malik, 1988). In addition, salt tolerance during accumulation of Na^+ and Cl^- at the cellular level can be achieved through loading in the vacuoles (Ashraf, 1994; Schachtman *et al*., 1995). Tomato (*Solanum lycopersicum* L.) is a short lived perennial, cropped as annuals. It belongs to the family Solanaceae (nightshade family) and is typically cultivated for its edible fruits. The leaves, stems and green unripe fruits of tomato plant contain small amount of the poisonous alkaloid tomatine (Barceloux, 2009). The levels of tomatine are generally too small to be dangerous; so foods such as fried green tomato are safe to eat. The majority of crop plants are relatively salt sensitive and are unable to tolerate high level of salinity (Levitt, 1980). Despite bulk data available on the effect of salt on tomato (Sanchez-Blanco *et al*., 1991; Fernandez-Garcia *et al*., 2004;

Amini and Ehsanpour, 2005; Amini *et al*., 2007; Shibli *et al*., 2007; Gumi *et al.,* 2012), not much has been done on Ion homeostasis of tomato. This research aimed at investigating the roles of ionic accumulation and balance in conferring salt tolerant trait of tomato (*Solanum lycopersicum* L.).

MATERIALS AND METHODS

This study was conducted in Biological Garden, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto-Nigeria. The seeds of tomato (*Solanum lycospersicum*) were obtained from a local market at Sokoto metropolis, Nigeria.

Plant Growth Condition

The seeds of *S. lycospersicum* were collected and surface sterilized by soaking in 5% sodium hypochlorate for 15 minutes and washed 3 times with sterile distilled water. The seed were first sown in a nursery bed and then uniformly germinated seedlings (10 days old) were selected and transferred to a polythene bag containing a mixture of river sand and manure (3:1 ratio). Sodium chloride (NaCl) was weighed and dissolved in irrigation water to make variant concentration of 50 mM, 75 mM and 100 mM of salt concentrations which were used to water the plants. The solutions were then stored in air tight cans to prevent evaporation which will in turns increase solution concentrations. The seedlings of tomato were divided into four groups: the first represented the control where no NaCl was added to the nutrient solution, the second, third and fourth groups received 50, 75 and 100 mM of NaCl treatments respectively, added to the nutrient solution. Each treatment was replicated three (3) times and each replicate consist of three (3) plants. The seedlings were exposed to varied salt concentration for 21 days.

Dry matter accumulation:

After 21 days of salt treatment, the seedlings were harvested and dry mass DM (g/plant) of *S. lycospersicum* was determined. Shoots and roots were left in desiccators at 80^{o}C for 2 days and parameters computed according the formula of Hunt (1981).

Elemental Analysis:

Dried plant material (0.2 g) was ashed in a muffle furnace at 500^{o}C for three (3) hours. The ashes were digested with 5 ml of 7N nitric acid (HNO_3). After appropriate dilution, the filtrate was assayed for Na^+ and K^+ using atomic flame emission spectrometry (Ranganna, 1977). From these results Na^+/K^+ levels was computed.

Statistical Analysis

The results were expressed as mean of three replicates $\pm$ standard deviation, and the data were subjected to analysis of variance (ANOVA) test. Differences between means were determined by LSD using MINITAB statistical software.

RESULTS AND DISCUSSION

The results on the effect of salinity on dry matter accumulation (g) of *S. lycospersicum* were presented in Table 1. Dry matter accumulation decreased with increased salt concentration in both shoot and root of tomato. However, the results differ significantly ($P<0.05$) both in shoot and root of *S. lycospersicum.* Salt stress inhibited dry matter accumulation of *S. lycospersicum* in the current study. Similar results was reported on

tomato by Amini *et al.* (2007) and Gumi *et al.* (2012); on finger millet by Manikandan and Designh (2009); on maize (*Zea mays*) by Mansour (1994) and Mansour *et al.* (2005) and on *Jatropha curcas* by Reddy *et al.* (2008). The amount of inorganic ions (Na^+ and K^+) increased with increased salt concentration (Figure 1 and 2). In roots of *S. lycospersicum*, Na^+ increased in a concentration dependent manner. i.e. 0 mM has 750 mg Kg^{-1} while 50, 75 and 100 mM has 1325, 1370 and 1375 mg Kg^{-1} respectively. In shoots, the magnitude of increase across treatments was higher with the control (0 mM) and 100 mM treatments recording 350 and 1625 mg Kg^{-1} respectively. Potassium (K^+) uptake decreased with increased salt stress concentration both in shoots and roots of the *S. lycospersicum* genotype studied. In roots, the control has 7000 mg Kg^{-1} and 100 mM has 3200 mg Kg^{-1}. In shoots, the salt treated groups (50, 75 and 100 mM) have 9750, 8500 and 550 mg Kg^{-1} respectively. Under salt stress, Na^+ competes with K^+ for uptake into roots through common transport systems and does this effectively since the Na^+ in saline environments is usually considerably greater than K^+ (Rains, 1989; Maathius *et al*., 1992). These findings can be attributed to the competitive interactions between K^+ and Na^+ ions and the inhibition of K^+ uptake by high concentration of Na^+ as reported by Bernstein, (1995). More than fifty (50) enzymes are known to be activated by K^+ and Na^+ cannot substitute in this role (Bhandal and Malik, 1988). Moreover, protein synthesis requires high concentration of K^+ for the binding of tRNA to ribosome (Blaha *et al*., 2000) and probably other aspects of ribosome functions (Wyn Jones *et al*., 1984).

A high cytosolic K^+/Na^+ ratio is important for maintaining cellular metabolism of plants under salt stress episode. In this study, the levels of Na^+ gradually increased with increasing concentration of salt, while K^+ levels decreased with increasing concentration of salinity stress. High levels of Na^+ inside the cell inhibits the uptake of K^+ thereby decreased K^+/Na^+ ratio which in turns affects plant metabolism (Maathius *et al*., 1992). The metabolic toxicity of Na^+ is largely due to its ability to compete with K^+ for binding sites essentials for cellular function. The maintenance of low cytosolic Na^+ concentration and high K^+/Na^+ homeostasis is an important aspect of salinity tolerance and that salt tolerant lines show higher K^+/Na^+ levels (Chattopadhyay *et al*., 2002). Based on the K^+/Na^+ ratio observed in this study, the *S. lycospersicum* variety studied could be classified as a salt-tolerant line. Cytotoxic ion (Na^+) was sequestered both in root and shoot of *S. lycospersicum* but higher concentration of Na^+ existed in shoot than in roots. The result coincided with previous reports by Ashraf (1994) and Schachtman *et al.* (1991) and (1995) who reported salt tolerance during Na^+ accumulation can be achieved through loading in the vacuoles via the vacuolar type H^+ -ATPase. Once Na^+ have entered the transpiration stream, they are transported into the leaves where the only possibility to counter their potential toxicity for cytosolic enzymes is the vacuolar sequestration. Infact, the concentration of Na^+ in the vacuole may exceed 2 to 5 folds of the cytoplasmic concentration (Yeo, 1999). This finding inferred that cytotoxic ions are compartmentalised both in shoot and roots at cellular level using selected antiporters and that more sequestration or loading was achieved in the leaves than in roots.

CONCLUSION

From these findings, Na^+ was sequestered both in shoot and roots by active antiporters to minimized cytosolic toxicity and that the ratio of Na^+ to K^+ (K^+ : Na^+) is a good indication of salt tolerant property due to the competitive interaction of these two ions. Based on

these, the *S. lycospersicum* studied is a salt tolerant line due to the high level of K^+ it accumulate even under salt stress episode hence accounted for its successful biomass accumulation at 50 mM salt concentration.

Table 1: Effects of salinity stress on dry matter accumulation of Shoots and roots of *S. lycospersicum*

Salt concentrations	Dry matter accumulation (g Plant^{-1})		
	Plant DW	**Shoot DW**	**Root DW**
0 mM	$2.0^a \pm 0.19$	$1.3^a \pm 0.13$	$0.7^a \pm 0.04$
50 mM	$2.1^a \pm 0.24$	$1.3^a \pm 0.13$	$0.8^a \pm 0.09$
75 mM	$1.4^b \pm 0.12$	$0.9^b \pm 0.08$	$0.5^b \pm 0.07$
100 mM	$1.0^c \pm 0.32$	$0.7^b \pm 0.18$	$0.4^b \pm 0.07$

*Means followed by the same superscript are the same.

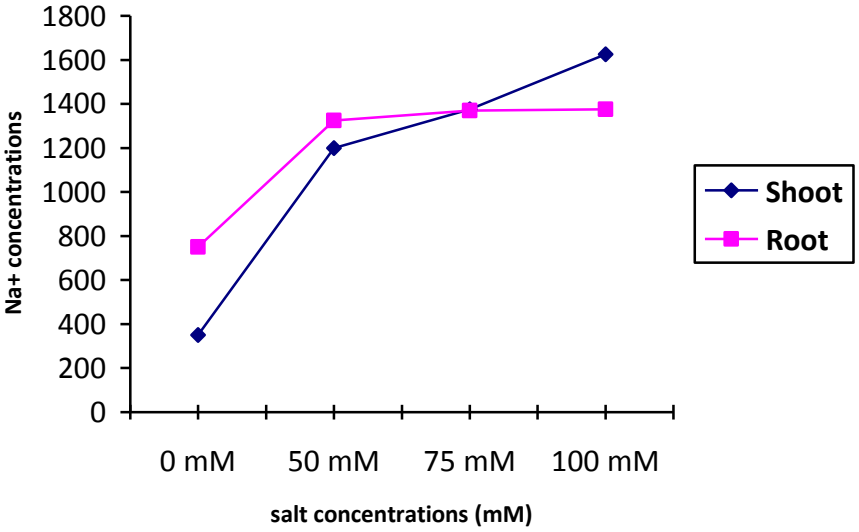

Figure 1: Sodium (Na^+) sequestration in shoot and roots of *S. lycospersicum* under different salt concentrations.

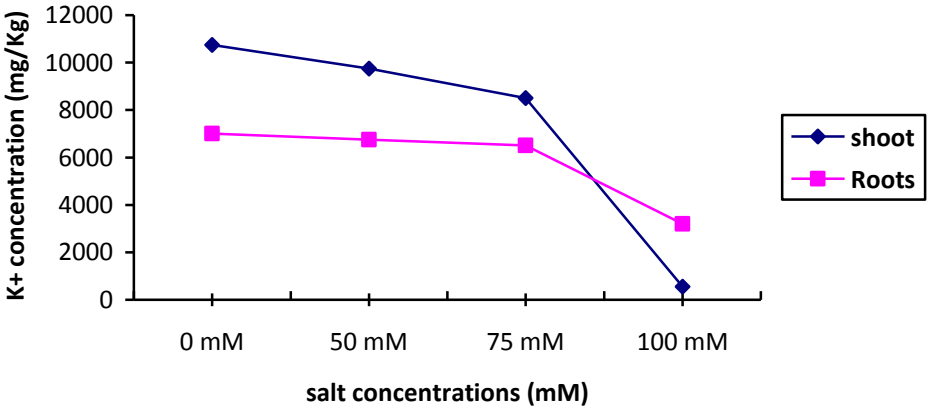

Figure 2: potassium (K^+) sequestration in shoot and roots of *S. lycospersicum* under different salt concentration.

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