AMELIORATION OF SALINITY INDUCE CHANGES IN TOMATO CROP GROWN IN SALINE CONDITIONS BY SUPPLEMENTAL POTASH APPLICATION

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***Abstract*-**The adverse effects of salinity are attributed to increased Na^+ and decreased K^+, Ca^{2+} and NO_3^-. Potassium being a major plant nutrient, salinity induced K^+ deficiency is a serious concerned. While the soil K may be adequate, the plants require additional Potash supplement under saline stress condition. In present study, the tomato plants were exposed to 0, 50, 100, 150 and 200 m*M* NaCl stress and supplemental potash (220 kg ha^{-1}) was applied from two sources *i.e.* Potassium Sulphate (SOP) and Potassium Chloride (MOP) against control (No supplemental potash). Application of supplemental potash at each levels of salinity, resulted in lower Na^+, higher K^+, thus lowered the Na^+/K^+ ratio, and decreased proline accumulation and improved the yield as well as lowered the blossom end rot incidence as compared to no supplemental potash treatments. While comparing the sources of supplemental K^+, SOP was more effective than MOP in enhancing K^+ accumulation, proline synthesis, ion leakage and decreasing blossom end rot incidence. It is concluded that the detrimental effects of salinity could be ameliorate through potassium sulphate supplementation in tomato crop.

***Index Terms*:** Salinity, Potassium Chloride, Potassium Sulphate, Proline, Ion Leakage.

INTRODUCTION

The tomato is an important vegetable crop and grown throughout the world. Tomato is a good source of nutrients especially Vitamin C and iron. Its regular consumption is believed to lower the risk of cancer (Etminan et al., 2004). The annual production of tomato is 529.6 thousand tons. Beside domestic consumption, Pakistan exported tomato worth Rs. 77 million during 2009-10 (MINFAL, 2012). The average yield of tomato is however; lower than International standards (Akhtar et al., 2010) due to biotic and abiotic stresses (Usman et al., 2013). Among the abiotic stresses, soil salinity is a major cause of lower yield (Oliveira et al., 2013). Salinity is a major abiotic stress that has adversely affected about 800 million hectares around the world (Shabala and Cuin, 2008). The performance of tomato crop is adversely affected by salinity levels above 2.5 dS m^{-1}, where each 1 dS m^{-1} increase in the salinity decreases plant growth by about 10% (Zayton et al., 2009). Salinity level of about 100 m*M* can decrease biomass production and yield (Saeed and Ahmad, 2009). The adverse effects of salinity are attributed to increased Na^+ and decreased K^+, Ca^{2+} and NO_3^- (Thalooth et al., 2006). The adverse effects of salinity can be decreased by removing the excess salts from the root zone by scraping, flushing and leaching (Siyal et al., 2002). However, less expensive methods such as foliar feeding of Fe, Zn and Mn have also been found to decrease the detrimental effects of excess Na^+ and Cl^- injury to plants (El-Fouly et al., 2002). Potassium (K) being a major plant nutrient, salinity induced K^+ deficiency is a serious concerned (Khan et al., 2006) that may disrupts metabolism and decrease growth and yield as well as fruit quality (Wang et al., 2013). The concentration of K^+ in the cytoplasm is between 100 and 200 m*M* (Sharma et al., 2013). However, apoplastic K^+ concentration could be as high as 500 m*M* (Wang et al., 2013). While the soil K may be adequate, the plants require additional K supplement under saline stress (Khan et al., 2006). The common sources of potash are Potassium Sulphate (SOP) and Potassium Chloride (MOP) and both have been found beneficial in promoting the uptake of other nutrients (Akhtar et al., 2010). Since, MOP and SOP with salt index of 116 and 46, respectively (Maynard and Hochmuth, 2007) can be used as K^+ source, it is needed to investigate the influence of supplemental potash and its sources on minimizing the adverse effects of salinity in tomato.

MATERIALS AND METHODS

Experimental Procedure

The effect of supplemental potassium on tomato growth and physiological changes was investigated by exposing tomato plants to 0, 50, 100, 150 and 200 m*M* NaCl and application of 220 kg/ha supplemental potash from two sources i.e. Murate of Potash (MOP), Sulfate of Potash (SOP) and control (no Potash) along with a basal dose of N 120 and P_2O_5 80 kg/ha as urea and triple super phosphate, respectively. The potassium dose of 220kg/ha was applied from both sources in two split doses; All P and half of N and K

fertilizers were manipulated in the media at the time of seed sowing and the remaining N and K fertilizers were applied at flower initiation stage as side dressing. The plants were harvested at the end of the growing season and the data were recorded on the following parameters.

Sodium Content

The sodium content of the tissue was determined using the methods of Watad et al. (1986). The tissue samples were oven dried at 80 ^{0}C up to the constant weight. The dried samples of shoots and roots were ground into a fine powder for wet digestion. For wet digestion, 5 ml of concentrated nitric acid were added to 0.2g of each ground sample. The samples were then kept at room temperature for 48 hours. On the next day the samples were placed in a hot-block set to 90 ^{0}C for approximately two hours. When no further color change was seen and sample particulates were no longer visible, the sample was removed from the hot block and allowed to cool and raised the volume of extract up to 50 ml by adding double distal water. The samples were then analyzed for sodium content by flame photometer (JENWAY PFP7).

Potassium Content

The potassium content of the tissue was determined using the methods of Watad et al. (1986). The same solution (as for sodium content) was used for the determination of potassium content in roots and shoots by flame photometer (JENWAY PFP7).

Sodium – Potassium ratio

Sodium and potassium ratio was calculated by dividing sodium content on potassium content values *i.e.*,

$$Ratio = \frac{Sodium\ \ Content}{Potassium\ \ Content}$$

Proline Content in shoot tips:

Proline was determined by the method of Bates et al. (1973). For this purpose 0.2g of fresh and young tips from each sample of shoot were taken and dip into liquid nitrogen for 2-3 minutes. The tissues were then crushed with a tissue miser and then homogenized with 4 ml of 3% sulfosalicylic acid (C_7 H_6 O_6 S.2H_2O). The homogenate was then centrifuged at 3000 rpm for five minutes at room temperature. The supernatant were filtered through Whatmann No. 2 filter paper and again mix a 4 ml of 3% sulfosalicylic acid. The filtrates were then reacting with 2cm^3 acid ninhydrin in a test tube in boiling water bath for one hour. Reaction was terminating in an ice bath. Reaction mixture was extract with 4cm^3 toluene and tubes were cool down to room temperature. Absorbance was measure at 520 nanometer against toluene blank.

Ion leakage

Five (17-mm diameter) leaf discs were punched from each plant with a cork borer. Punches avoided major veins. There were three replicate samples per treatment. The leaf discs were first left for 30 mints for healing and then placed in beaker containing 50 ml 0.3 molar Mannitol solution. The data recorded by conductivity meter (in mV). The data recorded after first 30 mints were considered as cell wall leakage and after 90, 120 and 180 mints were membrane leakage. The total leakage was measured by freezing and thawing three times the leaf discs from the same leaf for each replication. The percent membrane leakage was measured by the following formula:

$$Membrane\,Leakage = \frac{(L-180)-(L-30)}{Total\ \ Leakage}\,x100$$

Whereas, L_{180} is the leakage recorded after 180 mints and L_{30} is leakage recorded after 30 mints.

Blossom End Rot Incidence (%)

The incidence of blossom end rot (BER) was calculated by observing 30 fruits in each treatment and replication for the BER symptoms. The BER incidence is expressed as percentage of total fruits that had incidence.

Yield (t.ha^{-1})

All the marketable tomato fruit were weighted after picking and the total yield per plant was recorded in kilograms. Yield (tons per hectare) was then estimated from nine plants for each treatment.

Statistical Analysis

Statistical analysis was performed as for two factorial randomized complete block design (RCBD) (Steel and Torrie, 1980). The means were separated by the least significant difference (LSD) using MSTATC (Michigan State University, East Lansing, MI).

RESULTS AND DISCUSSION

Sodium Content

The sodium content of tomato leaves varied significantly with salinity levels and with potash sources (Table 1). The least sodium content (2103 µ*M*/g D.wt.) was recorded with 0 m*M* NaCl stress and supplemental potash from MOP, followed by 2151and 2326 µ*M*/g D.wt. with 0 m*M* NaCl stress + SOP and 0 m*M* NaCl stress + No supplemental potash treatment. Whereas, the Na^+ content of the tomato shoot increased at each level of salinity, the increase was less with MOP than SOP as a source of supplemental potash. Thus at the highest salinity level (200 m*M* NaCl), the Na^+ content of tomato leaves was 4269 and 4618 µ*M*/g D.wt. with MOP and SOP respectively, used as source of supplemental potash (Table 1). The uptake of sodium ions increases with increasing salinity levels (Juan, et al., 2005). Thus, the increased sodium content in leaves is one of the primary plant responses to salinity. The increased accumulation of Na^+ in tomato shoots and roots with increasing salinity stress, may cause ions toxicity (Juan et al., 2005), that is minimized by synthesizing compatible solutes (Flowers, 2004). The application of supplementary potassium is known to decrease the Na^+ and increase the K^+ content (Kaya et al., 2002). It is interesting to observe that MOP was more effective in decreasing the sodium content of the tomato shoot under salinity stress. Since, application of MOP may increases the Cl^- content of tomato as compared to SOP (Akhtar et al., 2010), it is likely that it interacts with other nutrients such as Na^+ and P and thus, decreases the adverse effects of salinity.

Potassium Content

The potassium content of tomato leaves decreased significantly with increase in salinity levels but the decline was lowered by supplemental potash application. The maximum K^+ content (4545 µ*M*/g D.wt.) was in non-stressed plants (0 m*M* NaCl), that decreased significantly with the increase of salinity levels and declined to the minimum (1617 µ*M*/g D.wt.) at 200 m*M* NaCl treatment. At each level of salinity supplemental Potash application resulted in high K^+ content as compared No supplemental potash treatments. However, the SOP source of potash was more effective in maintaining higher K^+ content. Thus, at 200 m*M* NaCl stress, the K^+ content was the highest (3520 µ*M*/g D.wt.) in SOP treatments, while it was 3225 and 1617 µ*M*/g D.wt. in plants supplied with MOP supplemental potash and No supplemental potash respectively (Table 1).

The uptake of K^+ is regulated by a K^+/Na^+ transporter in plants (Ashraf & Sarwar, 2002). Due to physicochemical similarities in Na^+ and K^+, Na^+ competes with K^+ for several binding sites, including K^+ transporter (Shabala & Cuin, 2008; Marschner, 2012). Thus, the potassium content of tomato shoot decreased with increasing levels salinity. In addition, high salinity disrupts membrane system leading to leakage of K^+ ions (Shabala & Cuin, 2008). Application of supplemental potash increased the K^+ content, indicating a positive effect on salinity induce decline in K^+ content (Akhtar et al., 2010). Since, adequate potassium content help in osmotic adjustment and maintenance of turgor at low leaf water potentials (Kaya et al., 2001), it may enhance salinity resistance, water use efficiency, plant growth and productivity under drought and salinity conditions (Marschner, 1995). However, the K^+ content was higher when supplemental potash was applied as SOP than MOP (Table 1).

Sodium Potassium Ratio

Salinity levels, potassium application and their interaction significantly influenced the Na^+/K^+ ratio in tomato leaves. The least Na^+/K^+ ratio in tomato plants exposed 0 m*M* NaCl stress ranged from 0.304 - 0.514, that increase with increasing salinity levels. In plants receiving no supplemental potash, the Na^+/K^+ ratio increased significantly with incremental increase in salinity and were the maximum of 3.303 in plants exposed to 200 m*M* NaCl stress. However, supplemental potash application lowered the increase in Na^+/K^+ ratio, so that it was 1.329 and 1.312 with supplemental potash application as MOP and SOP (Table 1).

The performance of tomato plants depends on an optimum Na^+/K^+ ratio. Generally, lower Na^+/K^+ ratio is required for maintenance of cell metabolism under salt stress. By contrast, high Na^+/K^+ ratio indicates ionic imbalance and may specific ion toxicity, leading to death of the tissue (Ashraf, 2004). The K^+ and Na^+ compete for uptake into the root (Hussein et al., 2007). Thus, salinity increases Na^+/K^+ ratio by increasing Na^+ and decreasing K^+ accumulation (Juan, et al., 2005). It leads to Na^+ toxicity if the access Na^+ is not transported to the vacuoles (Zhu, 2003), where it can be used as an osmotic solution (Yokoi et al., 2002). It is the reason that low rates of Na^+ transport and high selectivity for K^+ over Na^+ is correlated with salinity tolerance in plants (Sarwar & Ashraf, 2003). Application of supplemental potash decreased the Na^+/K^+ ratio. Both the K sources were significantly superior than no potash supplement but there was no significant difference between the two sources. Potash supplement enhances potassium uptake (Kaya et al., 2001) and a lower cytosolic Na^+/K^+ ratio. A high K^+ accumulation in plant tissue reduces the Na^+ concentration and results in a higher K^+/Na^+ ratio (Wang et al., 2013). The beneficial effects of potassium supplement as increased nutrient (K^+, Ca^{2+}, N, Mn^{2+} and Fe^{2+}) and plant growth have also been observed in water logging stress (Ashraf et al., 2011). While, sulphur application is known to decrease Na/K ratio (Abdelhamid et al., 2013), MOP application resulted in lower Na^+/K^+ ratio at each level of salinity.

Proline Content

The salinity stress and supplemental potash sources as well as the interaction of salinity and supplemental potash application significantly affected the accumulation of proline in the shoots of tomato plant. The least proline content (0.177 µ*M*/g F.wt.) in plants exposed to 0 m*M* NaCl and no supplemental potash treatment, increased significantly to 3.698 µ*M*/g F.wt. in plants exposed to 200

m*M* NaCl stress and no supplemental potash treatment. Whereas, the supplemental potash application from MOP source resulted in higher proline content (4.604 µ*M*/g F.wt.), it was lower (2.503 µ*M*/g F.wt.) with SOP treatment as compared to control treatment.

The plants synthesize a number of osmolytes such as proteins, carbohydrates, amino acids and proline in response to salinity stress (Ashraf & Harris, 2004). Salinity stress increases proline synthesis that enhances osmotic balance in the presence of excess Na^+ (Ashraf & Harris, 2004). Thus, the high proline content minimizes the adverse effects of salinity (Ali et al., 2011). Proline is also involved in scavenging hydroxyl radicals in plants exposed to drought and salt stress (Parida & Das, 2005). It seems that the salt stress up-regulated the enzymes involved in biosynthesis and elevated the levels of proline in the tissue (Munns, 2005). Exogenous proline application has also been found to decrease the damage due to high salinity (Deivanai et al., 2011). Potassium sources had a contrasting effect on proline content. While MOP resulted in 22.93% increase in proline content of the shoot, the SOP treatment declined it by 15.35%. It indicates that the influence of beneficial effect of supplemental potash is not mediated through proline synthesis (Babaeian & Ahmadi, 2002).

Membrane Leakage

The ion leakage from leaf discs in plants exposed 0 m*M* NaCl + no supplemental potash was 15.67%, which increased with increasing salinity levels and was the highest (29.33%) in plants exposed to 200 m*M* NaCl + no supplemental potash. Supplemental potash and its sources lowered the increased in ion leakage significantly. Thus, the ion leakage was 19.97 and 17.67% in plants exposed 0 m*M* NaCl + supplemental potash as MOP and SOP respectively. Similarly at the maximum salinity levels (200 m*M* NaCl stress), supplemental potash application from MOP and SOP resulted in 33.33 and 26.00% ion leakage from leaf discs of stressed plants. The ions leakage is common parameter of stress induced damaged, including salinity (Demidchik et al., 2014). Thus, ions leakage of tomato leaf discs used as measure of salinity induced changes in membrane permeability. It is suggested that the cell membrane stability is adversely affected by abiotic stresses (Wang & Huang, 2004) and membrane integrity and stability is essential for stress tolerance (Bajji et al., 2002). Salinity stress induces structural changes that increase membrane permeability (Kaya et al., 2002), due to enhanced lipids per oxidation (Kaya et al., 2006). Furthermore, salinity stress also induces drought stress in the tissue (Romero-Aranda et al., 2001). Thus, it is likely to observe increased membrane leakage with increasing salinity. Whereas salinity adversely affects cell membrane, potassium applications enhance cell membrane stability (Premachandra et al., 1991). By contrast, Sangtarashani et al. (2013) reported increased ion leakage with potassium application.

Table 1. Effects of potassium (MOP and SOP) potassium and potassium content, sodium potassium ratio and proline content of tomato plants grown in saline conditions.

Potash Source	Salinity Levels (m*M*)	Na^+ Content (µ*M*/g D.wt.)	K^+ Content (µ*M*/g D.wt.)	Na^+/K^+ Ratio	Proline Content (µ*M*/g F.wt.)	Ion Leakage (%)
Control	0	2326 $_{j}$	4545 $_{d}$	0.514 $_{hi}$	0.177 $_{n}$	15.67 $_{l}$
	50	3263 $_{g}$	3486 $_{ef}$	1.145 $_{de}$	1.513 $_{jk}$	23.00 $_{h}$
	100	4074 $_{de}$	2447 $_{g}$	1.67 $_{c}$	1.928 $_{gh}$	24.00 $_{gh}$
	150	4879 $_{b}$	2089 $_{g}$	2.346 $_{b}$	2.713 $_{d}$	27.00 $_{de}$
	200	5235 $_{a}$	1617 $_{h}$	3.303 $_{a}$	3.698 $_{b}$	29.33 $_{c}$
MOP	0	2103 $_{k}$	6931 $_{b}$	0.304 $_{j}$	0.993 $_{l}$	19.67 $_{j}$
	50	2635 $_{i}$	4690 $_{d}$	0.564 $_{gh}$	1.656 $_{ij}$	23.33 $_{h}$
	100	3626 $_{f}$	3603 $_{ef}$	1.015 $_{ef}$	2.027 $_{fg}$	27.33 $_{d}$
	150	4025 $_{e}$	3470 $_{ef}$	1.161 $_{de}$	3.05 $_{c}$	31.33 $_{b}$
	200	4269 $_{d}$	3225 $_{f}$	1.329 $_{d}$	4.604 $_{a}$	33.33 $_{a}$
SOP	0	2151 $_{jk}$	8416 $_{a}$	0.256 $_{ij}$	0.726 $_{m}$	17.67 $_{k}$
	50	2953 $_{h}$	5796 $_{c}$	0.509 $_{hi}$	1.322 $_{k}$	20.33 $_{j}$
	100	3879 $_{e}$	4838 $_{d}$	0.804 $_{fg}$	1.794 $_{hi}$	21.67 $_{i}$
	150	4519 $_{c}$	3695 $_{e}$	1.227 $_{de}$	2.144 $_{f}$	25.00 $_{fg}$
	200	4618 $_{c}$	3520 $_{ef}$	1.312 $_{d}$	2.503 $_{e}$	26.00 $_{ef}$
LSDs		203.0	444.0	0.2799	0.2048	1.208

Means followed by similar letters in a column are non significantly different from each other at α 0.05.

Yield (t. ha^{-1})

The yield of tomato declined significantly with increasing salinity, but the decline was less with supplemental potash application (Table 2). The yield (7.58 t. ha^{-1}) of control plants (0 m*M* NaCl stress + No supplemental potash) decreased significantly to 1.87 t. ha^{-1} at 200 m*M* NaCl level + No supplemental potash. While the decrease in yield due to salinity, were less with the application of supplemental potash at each salinity level. Comparing the sources of supplemental potash, SOP gave highest yield (8.12 t. ha^{-1}) at 0

m*M* NaCl stress and also at each level of salinity than MOP and no supplemental potash. Thus, the yield was 3.12 and 2.47 t. ha^{-1} in SOP and MOP treated plants respectively at 200 m*M* NaCl stress level. Salinity causes physiological drought which ultimately decreases the rate of fruit expansion (Johnson et al., 1992). The rate of photosynthates production (Tsonev et al., 2011) and transport of photosynthetic products in the plant system decreases in saline condition (Hajiboland et al., 2010). Thus, the low availability of photosynthates and water may causes reduction in yield (Juan et al., 2005; Rahman et al., 2006). Salinity also decrease potassium uptake (Wang et al., 2013) and its deficiency may also contribute to low production (Gong et al., 2011). Therefore, enhancing K availability through supplemental potash application may enhance photosynthates production and photo-assimilates translocation (Hartz et al., 1999). Potassium application may also increases the phosphorus content of tomato (Akhtar et al., 2010); this indicates a positive effect of supplemental potash on other nutrients uptake, which may synergistically increase the yield (Khan et al., 2006). The supremacy of SOP over MOP regarding yield, in the present study is in agreement with the findings of Loch & Petho (1992) but contrary to kiviani et al. (2004) in field condition. The differences in response of tomato to potassium sources could be due to cultivars of tomato understudy or the soil and climatic condition (Akhtar et al., 2010).

Blossom End Rot Incidence (%)

The blossom end rot incidence (33%) of control plants (0 m*M* NaCl stress + No supplemental potash) decreased significantly to 30 and 23% with supplemental potash application from MOP and SOP sources respectively. The blossom end rot increased with incremental increase in salinity and was the highest (63%) in plants exposed to 200 m*M* NaCl stress + No supplemental potash. By contrast the blossom end rot decreased with supplemental potash application from MOP and SOP. The increase in blossom end rot incidence at each salinity level was less with supplemental potash from SOP than MOP source. Thus, the blossom end rot incidence was 57 and 53% in plants exposed to 200 m*M* NaCl + supplemental potash from MOP and SOP sources respectively (Table 2). The blossom end rot of tomato is a mineral deficiency related physiological disorder and its incidence is correlated with deficiency of calcium and potassium (Taylor et al., 2004). Salinity declines nutrients uptake and disrupt K^+/Na^+ and Ca^{+2}/Na^+ balance (Magan et al., 2008) and lower calcium promote blossom end rot (Rab & Haq, 2012). The blossom end rot incidence is increased with increasing salinity (Aktas et al., 2003). Potassium application to plants grown in saline conditions may ameliorate the negative effect of NaCl (Kaya et al., 2002). It has been found that foliar application of Calcium (Schmitz-Eiberger et al., 2002) and potassium solutions (Peyvast et al., 2009) may decrease blossom end rot incidence. Thus, application of supplemental potash decreased the blossom end rot incidence (Zayton et al., 2009).

Table 2. The influence of salinity and supplemental potash on the yield and blossom end rot incidence of tomato fruit.

	Yield ($t.ha^{-1}$)				Blossom End Rot (%)			
Salinity Levels (m*M*)	Control	MOP	SOP	Means	Control	MOP	SOP	Means
0	7.58 $_{b}$	6.99 $_{c}$	8.12 $_{a}$	7.56	33 $_{de}$	30 $_{ef}$	23 $_{gh}$	29
50	6.74 $_{d}$	6.89 $_{cd}$	7.55 $_{b}$	7.06	23 $_{gh}$	23 $_{gh}$	17 $_{i}$	21
100	4.41 $_{h}$	4.97 $_{f}$	5.78 $_{e}$	5.05	33 $_{de}$	27 $_{fg}$	20 $_{hi}$	27
150	3.57 $_{i}$	4.21 $_{h}$	4.67 $_{g}$	4.15	47 $_{c}$	37 $_{d}$	27 $_{fg}$	37
200	1.87 $_{l}$	2.47 $_{k}$	3.12 $_{j}$	2.49	63 $_{a}$	57 $_{b}$	53 $_{b}$	58
Means	4.83	5.11	5.85		40	35	28	

Means followed by similar letters are non significantly different from each other at α 0.05
LSD for Interaction of salinity and K-Source for yield = 0.2116
LSD for Interaction of salinity and K-Source for BER = 5.582

CONCLUSION

On the basis of results obtained, it has been concluded that application of supplemental potash at each levels of salinity resulted in lower Na^+ concentration and enhanced K^+, thus lowered the Na^+/K^+ ratio and decreased proline accumulation, which ultimately improved the yield as well as lowered the blossom end rot incidence. While, among the sources of supplemental K^+, SOP was more effective than MOP in enhancing K^+ accumulation, proline synthesis, ion leakage and decreasing blossom end rot incidence. Therefore the detrimental effects of salinity could be ameliorating through potassium sulphate supplementation in tomato crop.

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