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MORPHO-PHYSIOLOGICAL RESPONSES OF MAIZE (*ZEA MAYS* L.) TO EXOGENOUS SALICYLIC ACID APPLICATION UNDER SALINITY STRESS

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Abstract

The effect of salicylic acid (SA) (0, 1 and 2 mM) on growth parameters and some physiological characteristics of three maize varieties (S.C704, S.C302 and S.C700) was investigated under salinity stress treatments (0, 50 and 100 mM NaCl). Experimental design was factorial split plot based on completely randomized block design with three replications. Results showed that with increasing salinity to 50 and 100 mM, increased SOD in most varieties. The most of superoxide dismutase activity was measured in S.C302 in 100 mM salt and 2 mM salicylic acid. Salicylic acid enhances the activity of the superoxide dismutase. In S.C704 salinity decreased catalase enzyme activity, but with the use of salicylic acid increased the activity of catalase. By the application of salicylic acid, ascorbate peroxidase enzyme activity increased in all varieties, while salinity reduced enzyme activity. Salinity decreased chlorophyll 'a' content in all varieties. Salinity causes the accumulation of sodium ions in the leaves and roots of the all variety. In S.C704 leaves the most sodium ions was found in 100 mM salt, and salicylic acid reduced the salt accumulation in leaves.

Introduction

In the current global climate change scenario one expected threat is the increase in land salinization (FAO 2011). Over 6% of the world's land is affected by salinity and its extent is increasing regularly throughout the world (Schwabe *et al.* 2006). Nowadays, high salinity in soils is a very serious problem for crop production because most of the cultivated plants are sensitive to salt stress (glycophytes) (Munns and Tester 2008).

One of the common responses to different environmental stresses, both abiotic and biotic, is the accelerated generation of reactive oxygen species (ROS), including superoxide ($O_2^{\bullet -}$), perhydroxy radical ($HO^{2\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), alkoxy radical ($RO^{\bullet}$), peroxy radical ($ROO^{\bullet}$), singlet oxygen (O_2), organic hydroperoxide (ROOH), and so forth (Bhattacharjee. 2010, Miller *et al.* 2008, Gill and Tuteja 2010, Miller *et al.* 2009). Accumulation of ROS imposes ultimately oxidative stress, exacerbating cellular damages (Kovalchuk. 2010). They constantly sense the level of ROS and reprogram their gene expression to respond to changes in their environment (Miller *et al.* 2010). The most common mechanism to detoxify ROS produced during salt stress response is the induction of ROS-scavenging enzymes, such as superoxide dismutase (SOD) and catalase (CAT). SOD converts $O_2^{\bullet -}$ to H_2O_2 and then CAT converts H_2O_2 to water and molecular oxygen in peroxisomes.

Salicylic acid (SA), which is an endogenous plant growth regulator and influences different physiological and biochemical functions in plants, is a well-known and naturally occurring signaling molecule that has important role in establishing and signaling a defense response against various biotic and abiotic stress (Arfan *et al.* 2007, Wang *et al.*2010). Plant physiological processes, growth, development, productivity, and responses to abiotic stresses are affected by SA application (Arfan *et al.* 2007). Hayat *et al.* (2010) reported that exogenous application of SA

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affect productivity, growth, photosynthesis, plant water relations, and antioxidant enzyme activities of plants exposed to various biotic and abiotic stresses. SA effectively alleviated the toxic effects generated in plants due to the exposure to various abiotic stresses (Hayat *et al.* 2010). SA enhanced the leaf area and dry matter production in corn and soybean (Khan *et al.* 2003), *Brassica juncea* (Fariduddin *et al.* 2003), wheat (Hayat *et al.* 2005, Hussein *et al.* 2007).

Maize (*Zea mays* L.) is one of the most important crops both for human and animal consumption. This crop is cultivated on more than 142 million ha of land worldwide and it is estimated to produce around 913 million tons of grain in the period 2012/2013 (IGC. 2012) accounting for one third of the total global grain production (Heng *et al.* 2009).

Materials and Methods

The study was began in spring of 2014 in the region of Ardabil branch Islamic Azad University by geographical coordinates 48 and 30 of east length and 38 and 15 of North width with the height of 1350 meter above sea. The climate of this region was cold and semidry. It has a long dry season especially in summer. The soil of the region is clay that is poor with respect of organic material. For the purpose of investigating all factors, the analysis of soil was conducted, to determine the limiting factors of growth in pot and to be sure that there is no primary limit in EC.

Table 1. Chemical and physical characteristics of the experimented pot soil.

Pwp (%)	FC (%)	Silt (%)	Sand (%)	Clay (%)	Pota-ssium ppm	Phos-phorus ppm	Organic carbon (%)	Organic carbon (%)	Neutral respon-dents (%)	Total acidity saturated	Ec mmols/cm	SP (%)
18	30	36	15	49	453	9.3	0.091	0.86	13.3	7.8	0.52	46

Three cultivar of maize including S.C704, S.C302 and S.C700 on zero salinity level (control), 50 and 100 mM of chloride sodium and three salicylic acid levels Zero (Control), 1 and 2 mM were cultivated on pot coincidently of the form of factorial split plot test on 3 replications. For the aim of growth limiting factors determination, the per cent of its juice was determined and the amount of the needed salt to reach to investigate salinity was calculated by using saltcalc software. The test was done by using plastic pots of dimensions of 25 × 35 that all of them were filled with leaf soil and sandy soil and fertilizer and cultivated soil. The cultivation was done on 3.5.2014. Irrigation was conducted on normal environmental condition. Foliar application with salicylic acid in two stages, 5 and 10 leaves was conducted. During the experiment, chlorophyll a, carotenoids, Na^+ in leaf, Na^+ in Root, K^+ in leaf, K^+ in root superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) and proline were measured. Leaves samples were immediately frozen in liquid nitrogen and stored at –80°C until analysis. For determination of antioxidant enzyme activities, fresh leaf samples (0.3 g) from control and treated plants were suspended in specific buffer and pH for each enzyme extraction. The homogenates were centrifuged at 14.000 rpm for 20 min at 4°C and resulting supernatants were used for enzyme assay. Superoxide dismutase (SOD) activity was determined based on the inhibition of education of nitro-blue tetrazolium in the presence of riboflavin in the light at 560 nm as described by Giannopolitis and Ries (1977). A unit of SOD activity is defined as the amount of enzyme, which caused 50% inhibition of the reaction in the absence of enzyme. Catalase activity was measured titrimetrically by Chance and maehly method (1955), whereas, peroxidase activity was measured on colorimeter, using purpurogallin for standard curve. The activity of APX was assayed according to Chen and Asada method (1992). The reaction mixture (3 ml) contained 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.5 mM H_2O_2 and 0.1 ml enzyme extract. The reaction was started by the addition

of H_2O_2. The activity of enzyme was assayed by measuring the decrease in absorbance at 290 nm for 1 min of ascorbic as ascorbic acid oxidized.

Photosynthetic pigments were measured using the method of Arnon (1975) and Ashraf *et al.* (1994). Leaf samples (0.5 g) were homogenized with acetone (80% v/v), filtered and make up to a final volume of 5 ml. Pigment concentrations were recorded from the absorbance of extract at 663 and 470 nm (for carotenoids). Finally, the results were expressed as milligram of pigment per gram of leaf fresh weight. Free proline accumulation was determined using the method of Bates *et al.* (1973). 0.04 gram dry weight of leafs was homogenized with 3% sulfosalicylic acid and after 72 hrs that proline was released; the homogenate was centrifuged at 3000 g for 20 min. The supernatant was treated with acetic and acid ninhydrin, boiled for 1 hr and then absorbance at 520 nm was determined by UV-visible spectrophotometer.

In order to perform ion plant tissue analysis, dry method was used. The amount of sodium and potassium ions in plant tissues were determined by using a Flame photometer (Jenway, England) in Hamada and EL-enany method (1994).

Statistical analysis was performed by Mstat software according to a factorial split plot design on the basis of RCBD. Means were compared by Duncan test at a significance level of 0.05 probability level. From the data collected, charts and curve fittings were performed using Microsoft Excel software.

Result and Discussion

Analysis of variance for the studied traits is shown in Table 1. For ascorbate peroxidase, superoxide dismutase, catalase, chlorophyll a, carotenoid, Na+ in leaf, Na+ in root, K+ in leaf, K+ in root and proline at different levels of salinity was a significant difference. We found that the activity of SOD was increased in the treatment groups compared to the control, regardless of SA treatment ($p < 0.05$). Azooz *et al.* (2009) in the activity of antioxidant enzymes in maize cultivar showed that in the control (no salt) with salinity levels significant differences was seen (Azooz *et al.* 2009). Drought and salinity stresses are two of the most important environmental factors limiting plant growth and productivity worldwide (Krasensky *et al.* 2012). The tolerance to salinity or water stress could be related to different genetically determined capacity of plants to cope with oxidative stress events (Golldack *et al.* 2011). Therefore, the identification of physiological and biochemical components of the antioxidative defense system, which have a potential to confer drought or salinity tolerance, could be essential for the characterization of stress tolerant plant species (Vaseva *et al.* 2011).

Activities of both CAT and SOD showed dramatic increases in plants under moderate salt stress (150 mM NaCl) compared with control, similar to findings by Barakat (2011). Between the varieties for SOD activity, chlorophyll 'a' and proline significant difference was seen in 1% probability level. The activity of superoxide dismutase in three varieties increased with increasing salinity and salicylic acid. The most of superoxide dismutase activities were measured in S.C302 in 100 mM salt and 2 mM salicylic acid. Salicylic acid enhances the activity of the superoxide dismutase (Table 3). Koca *et al.* (2007) and Athar *et al.* (2008) showed that superoxide dismutase activity in resistant varieties to salinity increases sharply.

In S.C704 salinity decreased catalase enzyme activity, but with the use of salicylic acid increased the activity of catalase. The highest catalase activity was observed at 50 mM salinity and 2 mM salicylic acid. In S.C700 salinity of 100 mM increased catalase activity, but with the use of salicylic acid increased the amount of activity.

The highest activity of catalase enzyme activity was observed at 100 mM salinity and 2 mM salicylic acid in S.C 302 and S.C700 varieties. Tuna *et al.* (2008) in effect of gibberellic acid and

salinity on antioxidants and growth parameters on corn plant showed that with increasing salt concentration, a significant decrease in dry weight, relative amount of chlorophyll and leaf water content was seen. By application of salicylic acid, ascorbate peroxidase enzyme activity increased in all varieties, while salinity was reduced enzyme activity. Most activity of ascorbate peroxidase (APX) in S.C302 in 50 mM salt and 2 mM salicylic acid was observed, that showed significant

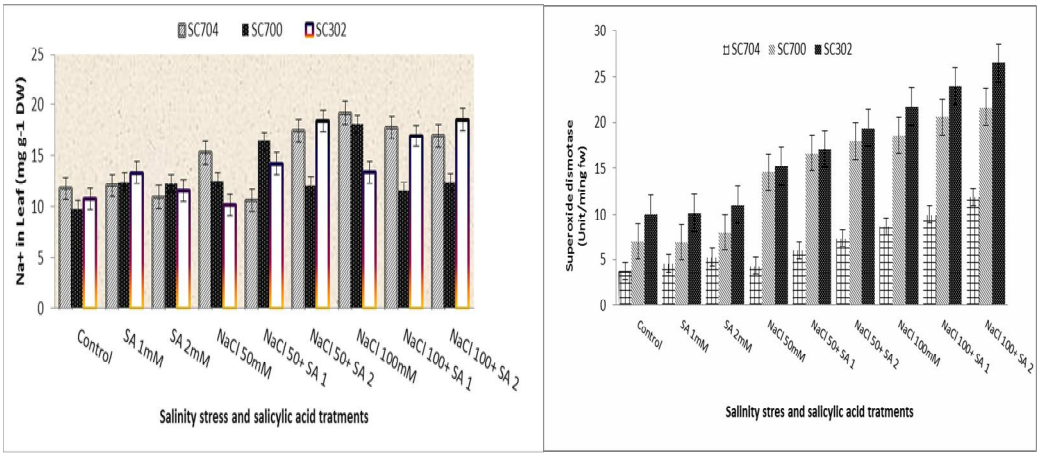

Fig. 1. Interaction effects of salinity stress and salicylic acid on superoxide dismutase and Na^+ in three maize varieties.

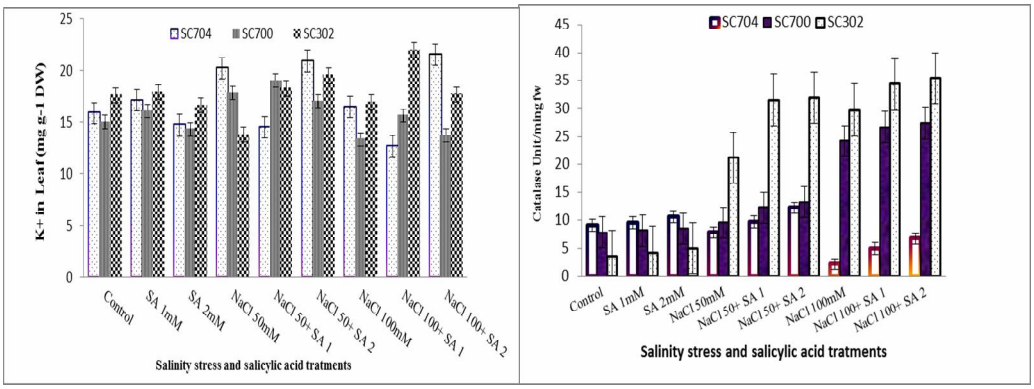

Fig. 2. Interaction effects of salinity stress and salicylic acid on potassium ion in leaves of three maize varieties.

differences with S.C700 and S.C704 varieties. Previous authors have also found that dark color is associated to higher antioxidant capacity (Lopez-Martinez *et al.* 2012, Hu and Xu 2011). Salinity decreased chlorophyll 'a' content in all varieties. The highest of chlorophyll 'a' content observed in control condition, which showed significant differences with all treatments. This is in line with results reported in sunflower (Manivannan *et al.* 2007), rice (Pattanagul 2011), bean (Dolatabadian and Jouneghan 2009), maize (Dolatabadian *et al.* 2009) and wheat (Moaveni 2011). Salinity and

Table 2. Analysis of variance of understudy characteristics in eight varieties of the maize.

Source	DF	Mean square									
		SOD	CAT	APX	Caro	Chl. a	Na^+ in leaf	Na^+ in root	K^+ in leaf	K^+ in root	Proline
Rep	2	0.928**	0.142**	0.142**	0.142**	0.018	0.008	0.032	0.016	0.045	0.0001
Salinity	2	0.646**	0.349**	0.349**	0.349**	0.212**	0.763**	1.098**	1.304**	0.778**	0.011**
SA	2	0.043*	0.029ns	0.029ns	0.029ns	0.078**	0.087 ns	0.044 ns	0.089 ns	0.005 ns	0.011**
SA*Na	4	0.001ns	0.055*	0.055*	0.055*	0.078**	0.046 ns	0.052 ns	0.065 ns	0.034 ns	0.002ns
Error	16	0.011	0.021	0.021	0.021	0.007	0.050	0.075	0.047	0.023	0.002
G	2	0.345**	0.009ns	0.009ns	0.009ns	0.006ns	0.014 ns	0.031 ns	0.016 ns	0.008 ns	0.004*
Na*G	4	0.026ns	0.016ns	0.016ns	0.016ns	0.006ns	0.026 ns	0.013 ns	0.031 ns	0.014 ns	0.003*
SA*G	4	0.001 ns	0.010ns	0.010ns	0.010ns	0.003ns	0.128*	0.064 ns	0.105*	0.037 ns	0.001ns
Na*SA*G	8	0.0001 ns	0.017ns	0.017ns	0.017ns	0.005ns	0.080 ns	0.102 ns	0.089*	0.073*	0.001ns
Error	36	0.038	0.011	0.011	0.011	0.006	0.041	0.053	0.046	0.027	0.001
	CV%	16.57	19.94	9.34	30.51	28.88	18.40	20.14	18.37	16.36	3.96

*Significant difference in probability level of 5%. **Significant difference in probability level of 1%. Rep: Replication, SA: Salicylic acid, Na: Salinity, G: Genotype, CV: Coefficient of variation, SOD: Superoxide dismutase,

Table 3. Influence of salinity stress and salicylic acid on superoxide dismutase, Catalase, ascorbate peroxidase, chlorophyll 'a' and proline of the three maize varieties.

Treatment	SOD unit/ming fw	CAT unit/ming fw	APX unit/ming fw	Chlorophyll "a" mg/g fw	Proline µmol/gfw
"S.C 704"					
Control	0.8494 fgh	0.5520a	9.463 ef	9.047 cde	3.700 d
SA 1 mM	1.596 abcdefg	0.1947cd	9.337 ef	9.477 bcde	4.553 d
SA 2 mM	1.750 abcdef	0.2303bcd	10.41 ef	10.45 bcde	5.230 cd
NaCl 50 mM	2.374 ab	0.2797bcd	9.463 ef	7.750 cde	4.317 d
NaCl 50 + SA 1	2.504 a	0.2467bcd	11.20 cdef	9.633 bcde	5.980 cd
NaCl 50 + SA 2	1.952 abcde	0.2137bcd	12.22 bcdef	12.15 abcde	7.317 bcd
NaCl 100 mM	1.238 cdefgh	0.1800d	8.533 f	2.123 e	8.640 bcd
NaCl 100 + SA 1	1.397 bcdefgh	0.1610d	10.81 def	4.910 de	9.953 abcd
NaCl 100 + SA 2	1.564 abcdefg	0.2440bcd	12.70 bcdef	6.687 cde	11.92 abcd
"S.C 700"					
Control	0.6208 gh	0.5883a	6.680 f	7.833 cde	7.020 bcd
SA 1mM	0.9723 efgh	0.3073bcd	7.050 f	8.080 cde	6.933 bcd
SA 2mM	1.606 abcdefg	0.3423bc	7.290 f	8.400 cde	7.990 bcd
NaCl 50mM	1.206 cdefgh	0.2267bcd	7.237 f	9.480 bcde	14.59 abcd
NaCl 50 + SA 1	1.387 bcdefgh	0.2587bcd	10.42 ef	12.17 abcde	16.66 abcd
NaCl 50 + SA 2	1.577 abcdefg	0.2377bcd	11.08 cdef	13.19 abcde	17.95 abcd
NaCl 100mM	0.9737 efgh	0.2167bcd	6.403 f	24.19 abcde	18.59 abcd
NaCl 100 + SA 1	2.104 abc	0.1993bcd	8.350 f	26.67 abcde	20.60 abcd
NaCl 100 + SA 2	1.977 abcd	0.1813d	11.32 cdef	27.30 abcde	21.67 abc
"S.C 302"					
Control	0.5436 h	0.5177a	18.37 abc	3.560 e	10.06 abcd
SA 1mM	0.9906 defgh	0.3530b	17.88 abcd	4.193 e	10.17 abcd
SA 2mM	1.556 abcdefg	0.2397bcd	18.82 ab	4.923 de	11.06 abcd
NaCl 50mM	1.307 cdefgh	0.2653bcd	16.42 abcde	21.13 abcde	15.28 abcd
NaCl 50 + SA 1	1.474 bcdefgh	0.2390bcd	19.33 ab	31.48 abc	17.11 abcd
NaCl 50 + SA 2	2.133 abc	0.2380bcd	21.21 a	31.90 abc	19.40 abcd
NaCl 100mM	1.256 cdefgh	0.1693d	8.350 f	29.68 abcd	21.72 abc
NaCl 100 + SA 1	1.487 bcdefgh	0.1783d	10.12 ef	34.26 ab	23.94 ab
NaCl 100 + SA 2	1.538 abcdefg	0.2497 bcd	12.46 bcdef	35.39 a	26.49 a

Different letters indicate significant differences for similar plant organs by Duncan tests at $p < 0.05$.

salicylic acid increased proline content in all varieties. In S.C704 the most of proline was obtained in 50 mM of NaCl and 1 mM salicylic acid. Proline content increased significantly under drought and severe salt stress conditions in *A. altissima* seedlings, supporting its role as a protective agent under oxidative stress conditions (De Carvalho *et al.* 2013). In S.C700 maximum of proline content was seen in 100 mM salt and 1 mM salicylic acid but in S.C302 the most of proline

content was seen in 50 mM salt and 2 mM salicylic acid, which with majority of treatment was significant differences. Salinity was reduced carotenoids in all varieties. No significant difference was observed between the control and application of salicylic acid in 700 varieties. The greatest amount of carotenoids was found in control condition in S.C302. Salinity causes the accumulation of sodium ions in the leaves and roots of the all variety.

Table 4. Influence of salinity stress and salicylic acid on carotenoids, Na^+ in leaf and root and K^+ in leaf and root of the three maize varieties.

Treatment	Carotenoids mg/gfw	Na^+ in leaf (mg/g DW)	Na^+ in root (mg/g DW)	K^+ in leaf (mg/g DW)	K^+ in root (mg/g DW)
"S.C 704"					
Control	17.45 ab	15.86 bcdefg	15.36 ab	11.82bcdef	0.559ab
SA 1 mM	8.640cde	17.10 abcdefg	15.93 ab	12.10abcdef	0.270defg
SA 2 mM	8.380cde	14.72 defg	16.62 ab	10.97cdef	0.399bcdef
NaCl 50 mM	7.893cde	20.22 abcd	20.08 ab	15.30abcdef	0.380bcdef
NaCl 50 + SA 1	8.387cde	14.50 defg	17.78 ab	10.63def	0.3137defg
NaCl 50 + SA 2	8.257cde	20.89 abc	21.90 a	17.48abcd	0.3407cdefg
NaCl 100 mM	7.683cde	16.46 abcdefg	18.35 ab	19.20a	0.2227fg
NaCl 100 + SA 1	10.35cde	12.69 g	17.70 ab	17.80abc	0.2013fg
NaCl 100 + SA 2	6.363cde	21.52 ab	18.50 ab	16.97abcde	0.3183defg
"S.C 700"					
Control	7.540cde	15.03 cdefg	13.98 ab	9.780f	0.6327a
SA 1 mM	11.51cd	16.04abcdefg	18.19 ab	12.48abcdef	0.4440abcde
SA 2 mM	17.89a	14.32 defg	16.37 ab	12.29abcdef	0.5407abc
NaCl 50 mM	7.667cde	17.85 abcdefg	14.26 ab	12.53abcdef	0.3010defg
NaCl 50 + SA 1	10.36cde	19.04 abcdef	17.58 ab	16.43abcdef	0.3623bcdefg
NaCl 50 + SA 2	5.077de	17.05 abcdefg	12.35 ab	12.10abcdef	0.3223defg
NaCl 100 mM	6.933cde	13.35 fg	18.25 ab	18.15ab	0.2530efg
NaCl 100 + SA 1	9.343cde	15.63 bcdefg	19.73 ab	11.59bcdef	0.2310fg
NaCl 100 + SA 2	5.517de	13.74 efg	12.48 ab	12.46abcdef	0.2177fg
"S.C 302"					
Control	12.34bc	17.63 abcdefg	16.68 ab	10.78cdef	0.6043a
SA 1 mM	9.697cde	17.93 abcdefg	17.69 ab	13.36abcdef	0.4777abcd
SA 2 mM	4.720e	16.61 abcdefg	10.53 b	11.60bcdef	0.2597efg
NaCl 50 mM	8.590cde	13.82 efg	15.17 ab	10.21ef	0.3150defg
NaCl 50 + SA 1	5.603de	18.29 abcdefg	13.04 ab	14.24abcdef	0.3370defg
NaCl 50 + SA 2	5.737de	19.56 abcde	16.81 ab	18.44ab	0.3490cdefg
NaCl 100 mM	10.60cde	16.92 abcdefg	17.93 ab	13.38abcdef	0.1623g
NaCl 100 + SA 1	8.027cde	21.96 a	16.21 ab	16.97abcde	0.2070fg
NaCl 100 + SA 2	8.993cde	17.71abcdefg	15.93 ab	18.56ab	0.3337defg

Different letters indicate significant differences for similar plant organs by Duncan tests at $p < 0.05$.

In S.C704 leaves the most sodium ions was found in 100 mM salt, and salicylic acid reduced the salt accumulation in leaves. But no significant difference was found between concentrations of salicylic acid in the salinity 100 mM. Iqbal *et al*. (2014) have reported that SA application induced Na+ accumulation in leaves in 0.5 mM SA and 0.5 mM + 100 mM NaCl in mungbean (*Vigna*

radiata L.). In S.C704 leaves the most potassium ion was found in 100 mM salt and 2mM SA. Salicylic acid increased the K+ accumulation in leaves (Table 4).

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