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Effects of salicylic acid and salinity on growth of maize plant (*Zea mays* L.)

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Abstract

To evaluate the effect of salt stress and salicylic acid application on growth and physiological traits of maize varieties, an experiment was conducted in factorial split plot based on RCBD design with 3 replications in research farm of Islamic Azad University of Ardebil branch during 2012-13. Salt stress factor including three levels (control, 50mM and 100mM NaCl) and acid salicylic (control, 1mM and 2mM). Results from the experiment showed that, between different salinity between different salinity in carotenoid, chlorophyll a+b, chlorophyll content and proline were significantly different. Effect of salicylic except for stem diameter was not significant for all traits. Interactive effects of salinity in salicylic on proline was significant at the 5% level. There was no significant difference in leaf length between salinity, but salinity decreased the amount of leaf length was 100 Mm NaCl in soil. The lowest of Leaf length was observed at 100 mM salt and 2 mM salicylic acid concentration. Chlorophyll content was measured by chlorophyll meter showed that with increasing salinity, chlorophyll content also show a significant decrease. The maximum stem diameter at the concentration of 100 mM salt, and 1 mM salicylic acid was observed in 640Ns variety (26/83mm). The highest amount of carotenoids in the leaves of salt-zero (control) was observed with increasing salt were significantly lower. Between leaf length with chlorophyll content, total chlorophyll (a+b) and carotenoid was observed significant positive correlation. There was a significant positive correlation between chlorophyll content with total chlorophyll, carotenoids and stem diameter.

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Introduction

The world population is expanding rapidly and is expected to be around 8 billion by the year 2025 (Andersen *et al*, 1999). This represents an addition of nearly 80 million people to the resent population every year. It is forecast that the increase in world population will occur almost exclusively in developing countries, where serious nutritional problems exist at present, and population pressure on agricultural soils is already very high. Maize (*Zea mays* L.), the third most important cereal crop after wheat and rice, is the major crop in Iran. Yield of maize crop is sensitive to abiotic stresses and may cause more than 50% yield reduction (Cakmak, 2005). Soil or water salinity is known to cause considerable yield losses in most crops, thereby leading to reduced crop productivity (Ashraf, 2009; Cha-um *et al*, 2011). The salinity-induced crop yield reduction takes place due to a number of physiological and biochemical dysfunctions in plants grown under salinity stress which have been listed in a number of comprehensive reviews on salinity effects and tolerance in plants (Ashraf *et al*, 2008; Munns and Tester, 2008; Jamil *et al*, 2011; Krasensky and Jonak, 2012). Scientists have been vying for the last many decades to overcome the problem of salinity by employing a variety of strategies. Of the various strategies currently under exploitation, improvement in salinity tolerance of crops through exogenous application of different types of chemicals including plant growth regulators, osmoprotectants and inorganic nutrients seems to be an efficient, economical and shot-gun approach (Ashraf *et al*, 2008). The use of such substances has resulted in a substantial increase in both growth and yield of many crops grown under saline conditions (Ashraf *et al*, 2008; Kaya *et al*, 2010). One mechanism utilized by the plants to overcome the salt stress effects might be via accumulation of compatible osmolytes, such as proline and soluble sugar (Shahba *et al*, 2010).

salicylic acid is an important commonly occurring signaling molecule in plants (Chen *et al*, 2009) response to adverse environmental conditions like low temperature (Ahmad *et al*, 2012; Farooq *et al*, 2008) and salinity stress (Khan *et al*, 2010). Exogenously applied salicylic acid helps plants to regulate several functions including systemic acquired resistance (SAR) and plant resistance to chilling stress in maize (Farooq *et al*, 2008). SA, an endogenous plant growth regulator, has been found to generate a wide range of metabolic and physiological responses in plants by affecting their growth and development (Hayat *et al*, 2010).

Increase in the efficiency of photosynthesis of maize under the influence of salicylic acid improves the plant's growth and yield (Khan *et al*, 2003). Application of 1 mM acid salicylic was reported to reduce transpiration. Spraying salicylic acid is also shown to be effective on the overall plant performance and its components (Azizi Yegane, 2010).

Therefore, the present investigation was undertaken to study the impact of spraying salicylic acid on some morphological and physiological characters of maize cultivars (*Zea mays* L.) in soil salinity condition.

Materials and methods

Location of test implementation

Investigate the effects of salt stress on some physiological and morphological traits in three varieties including (S.C580, NS640 and S.C704) and three salinity levels including Zero (control), 50 and 100 mM NaCl and three salicylic acid levels including Zero (control), 1mM and 2mM in three replicates for the factorial split plot experiment in randomized complete block design was carried out in research farm of Islamic Azad University of Ardebil branch during 2012-13. Treatments were planted in pots. Soil analysis of the experimental pots is presented in Table 1.

Mode of test implementation

During the experiment, several traits including Chlorophyll content, Carotenoid, Leaf length, chlorophyll a+b, proline and Stem diameter were measured. During the experiment, before dealing amount of proline, total chlorophyll Content was measured in the laboratory. Photosynthetic pigments (chlorophyll a and b) were measured using the method of Arnon (1975) and Ashraf (1994) in fresh leaf samples, a week before the harvest. One plant per replicate was used for chlorophyll determination. Prior to extraction, fresh leaf samples were cleaned with deionized water to remove any surface contamination. Leaf samples (0.5 g) were homogenized with acetone (80% v/v), filtered and make up to a final volume of 5 mL. Then the solution for 10 minutes away in 3000 (rpm) centrifuged. Pigment concentrations were calculated from the absorbance of extract at 663 and 645 nm using the formula given below:

a) Chlorophyll a (mg/g FW) = [12.7× (A663) _ 2.69× (A645)] ×0.5

b) Chlorophyll b (mg/g FW) = [22.9× (A645) _ 4.69× (A663)] ×0.5

Free proline accumulation was determined using the method of Bates *et al.,* (1975). 0.04 gram dry weight of leafs was homogenized with 3% sulfosalicylic acid and after 72h 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 hour and then absorbance at 520 nm was determined by Uv-visible spectrophotometer.

Statistical Analysis

Statistical analysis of the data was done on the basis of randomized complete block design. The average of attendances was calculated on the basis of Duncan method at 5% probability level.

Result and discussion

Analysis of Variance

Results from the experiment showed that, between different salinity in carotenoid, chlorophyll a+b, chlorophyll content and proline were significantly different. Effect of salicylic except for stem diameter was not significant for all traits. Interactive effects of salinity in salicylic on proline was significant at the 5% level. Between genotypes in the proline significant differences were found. Effect of interaction between salinity in genotype showed no significant difference for all traits. Interaction salicylic in the genotype was significant differences for proline. The coefficient of variation was calculated for all traits. The maximum coefficient of variation of the proline with 24.38% and the lowest in stem diameter with 11.01% was measured (Table 2).

Table 1. Physical and chemical soil analysis.

Pwp%	FC%	Silt%	Sand%	Clay%	Potassium ppm	Phosphorus ppm	N Total%	Organic carbon%	Percentage of neutral solutes	PH	Ec Mmohs/cm	%SP
18	30	36	15	49	453	9.3	0.091	0.86	13.3	7.8	0.52	46

Table 2. Analysis of variance on mean of squares of measured traits maize cultivar.

Source	DF	Mean Square					
		Carotenoid	Leaf length	Chlorophyll a+b	Chlorophyll content	Stem diameter	proline
Replication	2	0.097**	11.198ns	0.177**	0.816 *	0.399	0.142
Salinity	2	0.111**	88.383 ns	0.064*	5.830**	8.236ns	0.296*
SA	2	0.028ns	55.790 ns	0.027ns	0.307 ns	25.053**	0.066ns
SA*Salt	4	0.010ns	136.235 ns	0.008ns	0.235 ns	5.895ns	0.236 *
Error	16	0.014	64.295	0.015	0.207	3.974	0.082
Genotype	2	0.001ns	6.568 ns	0.002ns	0.679ns	4.678ns	0.393**
Salt*Var	4	0.003ns	4.346 ns	0.005ns	0.122ns	6.218ns	0.103ns
SA*Var	4	0.018ns	41.253 ns	0.019ns	0.154 ns	3.723ns	0.236 **
Salt*SA*Var	8	0.012ns	47.948 ns	0.021ns	0.149 ns	4.787ns	0.132 *
Error	36	0.011	73.636	0.019	0.427	6.228	0.058
	CV%	18.35	12.24	21.11	20.35	11.01	24.38

*Significant difference in probability level of 5% ** significant difference in probability level of 1%.

Table 3. Comparisons of average between concentrations of various salt and salicylic acid.

Salt *SA	Carotenoid mg/g FW		Leaf length (cm)		Chlorophyll a+b mg/g FW		Chlorophyll content mg/g FW		Stem diameter (mm)		Proline µmol/gFw	
0*0	0.4730	a	74.22	a	0.4460	a	13.71	a	23.10	abc	1.438	a
0*1	0.3972	ab	69.22	ab	0.3612	a	13.38	a	23.99	ab	1.355	ab
0*2	0.3998	ab	69.67	ab	0.3254	ab	13.41	a	22.17	bc	1.258	abc
50*0	0.3542	ab	68.22	ab	0.3498	a	11.88	ab	22.□9	bc	0.915	abc
50*1	0.3377	ab	72.22	a	0.3200	ab	10.52	abc	22.44	bc	0.5310	c
50*2	0.3373	ab	73.22	a	0.3021	ab	10.47	abc	21.28	c	1.176	ab
100*0	0.3364	ab	71.78	a	0.3082	ab	8.344	cd	21.62	c	1.156	ab
100*1	0.2034	c	69.44	ab	0.2081	b	9.029	bc	24.78	a	1.189	ab
100*2	0.2677	bc	62.78	b	0.2724	ab	6.311	d	22.19	bc	0.7002	bc

* Different letters indicate significant differences at the level of 5%.

Table 4. Comparisons of average between different concentrations of salicylic acid and variety.

Var *SA	Carotenoid mg/g FW		Leaf length (cm)		Chlorophyll a+b mg/g FW		Chlorophyll content mg/g FW		Stem diameter (mm)		Proline µmol.gFw	
Sc580*0	0.4341	a	71.44	a	0.4260	a	11.8	a	22.92	ab	1.605	a
Ns640*0	0.3556	ab	68.56	a	0.3476	ab	10.90	a	21.23	b	0.9136	b
Sc704*0	0.3740	ab	74.22	a	0.3304	ab	11.86	a	22.96	ab	0.9914	ab
Sc580*1	0.2520	b	70.56	a	0.2241	b	11.60	a	24.23	a	1.04	ab
Ns640*1	0.3458	ab	70.67	a	0.3352	ab	9.778	a	23.53	ab	0.9802	b
Sc704*1	0.3406	ab	69.67	a	0.3300	ab	11.55	a	23.44	ab	1.055	ab
Sc580*2	0.3527	ab	69.22	a	0.2766	ab	9.844	a	21.50	ab	1.384	ab
Ns640*2	0.3602	ab	69.33	a	0.3160	ab	8.522	a	21.78	ab	1.171	ab
Sc704*2	0.2919	b	67.11	a	0.3074	ab	11.82	a	22.36	ab	0.5793	c

*Different letters indicate significant differences at the level of 5%.

Table 5. Correlation coefficients between traits.

	Leaf length	Chlorophyll content	Stem diameter	Chlorophyll a+b	Carotenoid	Proline
Leaf length	1	0.307**	0.175	0.227*	0.266*	0.052
Chlorophyll content		1	0.331**	0.426**	0.475**	0.116
Stem diameter			1	-0.025	-0.063	0.239*
Chlorophyll a+b				1	0.938**	-0.010
Carotenoid					1	0.124

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Comparison of mean

The mean traits using Duncan's method at different of salinity showed that most traits are significant differences. Between genotypes was not significant difference in stem diameter between salt concentrations. There was no significant difference in leaf length between salinity, but salinity decreased the amount of leaf length was 100 Mm NaCl in soil. The lowest of Leaf length was observed at 100 mM salt and 2 mM salicylic acid concentration. Chlorophyll content was measured by chlorophyll meter showed that with increasing salinity, chlorophyll content also show a significant decrease. The lowest chlorophyll content at 100 mM salt with 7.895 was found to have significant differences with others treatments. The maximum stem diameter at the concentration of 100 mM salt, and 1 mM salicylic acid was observed in 640Ns variety (26/83mm). The highest amount of carotenoids in the leaves of salt-zero (control) was observed with increasing salt were significantly lower.

Parida and Das, (2005) reported that the relative water content, water potential and osmotic potential of plants become more negative with an increase in salinity. The amount of chlorophyll a with increase soil salinity showed a significant decrease compared to control. These results are in accordance with those of exogenously applied ascorbic acid, salicylic acid and hydrogen peroxide increased chlorophyll *a* in wheat (Khan, 2007; Wahid *et al*, 2007) and canola (Sakr and Arafa, 2009) under stressful conditions. Between of salinity 50 to 100 mM, a significant difference was not found. But the least amount of chlorophyll a in 100 mM of salinity with 0.2001 mg chlorophyll per g fresh weight of leaves was obtained. Khodary (2004) also reported a similar increase in the growth of shoots and roots of Maize plants in response to salicylic acid treatment. In soybean plants, treatment with salicylic acid, increased pigments content as well as the rate of photosynthesis (Zhao *et al*, 1995). Sinha *et al* (1993) pointed out that chlorophyll and carotenoid contents of maize leaves were increased upon treatment with SA. Molazem *et al* (2012) in study the effect of salt stress on the antioxidant enzyme activities on the leaves Maize in different of salinity showed that with increasing salinity, significant reduction in leaf relative water content was observed. Highest proline with 1.351 mmol g fresh weight of leaves was obtained in normal conditions. Sakhabutdinova *et al* (2003) reported that salinity and water deficit induces accumulation of proline in wheat plants. The interaction effects between salicylic in Genotype showed that most traits between varieties at different concentrations of salicylic acid, not seen significant differences. These results are in accordance with Shahba *et al* (2010), who found that SA decreased proline level in tomato under salinity stress. Foliar application of salicylic acid significantly increased yield and its components of maize (Abdel-Wahed *et al*, 2006) and wheat plants (Iqbal and Ashraf, 2006).

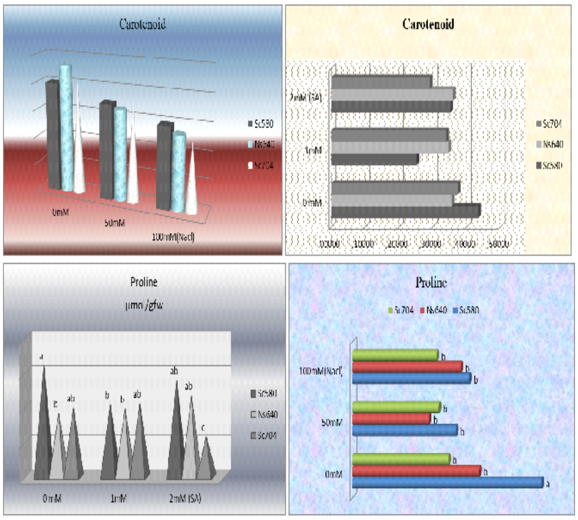

Fig. 1. Diagram of different understudy characteristics in three cultivars of the maize under the SA and salty conditions.

Simple correlation coefficients

Significant positive correlation between chlorophyll content with stem diameter, leaf length and carotenoids were observed. Significant positive correlation between stem diameter and proline was calculated. Correlation between the leaf length and carotenoids was positive and significant (table5). Between leaf length with chlorophyll content, total chlorophyll (a+b) and carotenoid was observed significant positive correlation. Similar results were also reported by Molazem and Azimi, (2011). There was a significant positive correlation between chlorophyll content with total chlorophyll, carotenoids and stem diameter. There was a significant positive correlation between stem diameter and proline.

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