Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 4, No. 5, p. 46-52, 2014
http://www.innspub.net

RESEARCH PAPER | OPEN ACCESS

Effect of exogenous application of salicylic acid on the growth, photosynthesis and proline content on maize in salt stress

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Article published on May 09, 2014

Key words: Salicylic acid, chlorophyll content, maize, salinity.

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 in plant height, chlorophyll a, leaf area index and proline were significantly different. Effect of salicylic except for chlorophyll a was not significant for all traits. With the increase of salt in the soil, plant height was significantly reduced. Minimum plant height in the third of salinity with 111 cm was obtained that with the dose of 50 mM had no significant difference. Leaf relative water content decreased with increasing salt, but this decrease was not significant. 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. Leaf area decreased with increasing salinity. Highest proline with 1.351 mmol g fresh weight of leaves was obtained in normal conditions. Between chlorophyll a with chlorophyll b, significant positive correlation was obtained. But with proline a non-significant negative correlation was found. Between leaf area index with leaf relative water content, chlorophyll a and chlorophyll b was seen a significant positive correlation

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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.) is considered as one of the most important cereal crops used in human consumption, animal feeding and starch industry and oil production (Amin *et al*, 2007). It is the most important cereal crop in the world after rice and wheat.

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).

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).

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.

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

Mode of test implementation

During the experiment, several traits including plant height, leaf relative water content (LRWC), chlorophyll a, chlorophyll b, proline and leaf area index were measured. During the experiment, before dealing amount of proline, chlorophyll a and Chlorophyll b Content were 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. leaf relative water content (LRWC) was calculated on the basis of Yamasaki and Dillenburg method (1999). Two leafs were randomly chosen from middle parts of the plants in each repetition. At first, leafs were separated from the stems and their fresh masses (FM) were calculated. In order to measure the saturation mass (TM), they were placed into the distilled water in closed containers for 24 hours under the air condition of 22° C, for the purpose of being reached to their greatest amount of saturation mass and then, they were weighed. Then leafs were placed inside the electrical oven for 48 hours under the air condition of 80° C and the dry mass of the leafs (DM) were obtained (DM). All of the measurements were done by scales with 0.001g accuracy and were placed into the following formula and into the following formula:

LRWC (%)= [(FM-DM)/(TM –DM)] ×100

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 plant height, chlorophyll a, leaf area index and proline were significantly different. Effect of salicylic except for chlorophyll a was not significant for all traits. Interactive effects of salinity in salicylic on plant height and 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. For proline, salicylic interaction in the genotype was significant differences. The coefficient of variation was calculated for all traits. The maximum coefficient of variation of the proline with 24.38% and the lowest in chlorophyll b with 4.72% was measured (Table 2).

Comparison of mean

The mean traits using Duncan's method at different of salinity showed that most traits are significant differences. With the increase of salt in the soil, plant height was significantly reduced. Minimum plant height in the third of salinity with 111 cm was obtained that with the dose of 50 mM had no significant difference. Leaf relative water content decreased with increasing salt, but this decrease was not significant. The results showed that SA spraying was improved chlorophyll content and RWC. 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. 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. Taking together, the results of the previous authors support our findings. Leaf area decreased with increasing salinity. Maximum leaf area in normal condition and the lowest amount of them in salinity 100 mM was calculated. 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. The interaction effects between salicylic in Genotype showed that most traits between varieties at different concentrations of salicylic acid, not seen significant differences. 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).

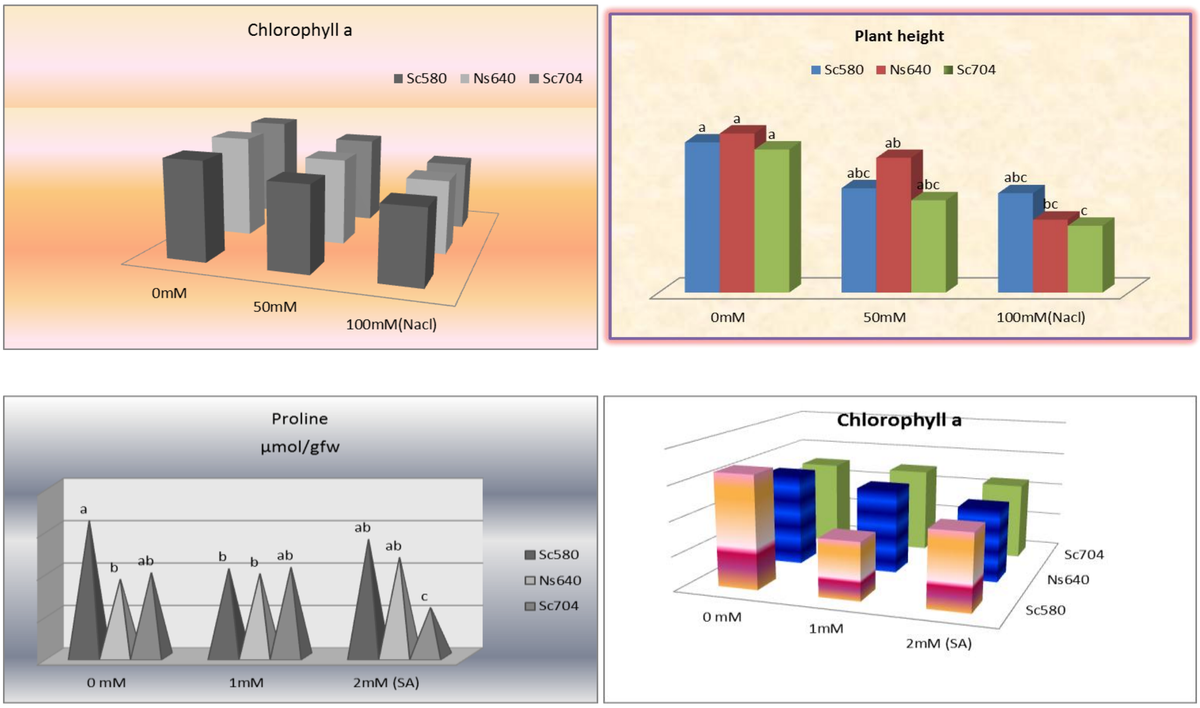

Fig. 1. Diagram of different understudy characteristics in three cultivars of the maize under the SA and salty conditions

Simple correlation coefficients

Between plants height with chlorophyll a and LRWC significant positive correlation was obtained. Similar results were also reported by Molazem and Azimi (2011). Between chlorophyll a with chlorophyll b, significant positive correlation was obtained. But with proline a non-significant negative correlation was found. Between leaf area index with leaf relative water content, chlorophyll a and chlorophyll b was seen a significant positive correlation (table5).

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

Source	DF	Mean Square					
		LRWC	Plant height	Chlorophyll a	Chlorophyll b	Leaf area	proline
Replication	2	14.887	537.827*	0.103**		8278.199	0.142 0.0014**
Salinity	2	36.246ns	1387.938**	0.036 *		32966.757*	0.296* 0.0002 ns
SA	2	23.081ns	109.827 ns	0.035 *		9795.963ns	0.066ns 0.0001 ns
SA*Salt	4	26.666ns	291.772*	0.013ns		5420.327ns	0.236* 0.0001 ns
Error	16	46.650	119.869	0.011		6644.509	0.082 0.0002
Genotype	2	29.366ns	142.309 ns	0.001ns		2618.791ns	0.393** 0.0001 ns
Salt*Var	4	7.708ns	82.642 ns	0.001ns		2247.162ns	0.103ns 0.0002 ns
SA*Var	4	9.067ns	242.920 ns	0.016ns		6125.494ns	0.236** 0.0001 ns
Salt*SA*Var	8	23.296ns	233.753 ns	0.009ns		2459.549ns	0.192* 0.0002 ns
Error	36	24.991	159.123	0.013		6279.304	0.058 0.0001
	CV%	6.42	10.70	23.49	4.72	17.49	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 salts and salicylic acid

Salt *SA	LRWC (%)		Plant height(cm)		Chlorophyll a mg/g FW		Chlorophyll b mg/g FW		Leaf area(cm²)		Proline µmol/gFw	
0*0	80.23	a	132.3	a	0.3538	a	0.09300	a	533.8	a	1.438	a
0*1	78.57	a	119.2	bc	0.2728	ab	0.08856	a	482.6	ab	1.355	ab
0*2	78.51	a	124.4	ab	0.2197	bc	0.1057	a	457.1	ab	1.258	abc
50*0	75.86	a	114.4	bcd	0.2572	ab	0.09256	a	450.5	ab	0.9153	abc
50*1	77.16	a	121.0	abc	0.2370	abc	0.08289	a	439.1	b	0.5310	c
50*2	77.45	a	116.2	bcd	0.2298	abc	0.07211	a	446.8	ab	1.176	ab
100*0	80.54	a	111.6	cd	0.2408	abc	0.06733	a	426.3	b	1.□56	ab
100*1	75.58	a	115.4	bcd	0.1534	c	0.05467	a	447.5	ab	1.189	ab
100*2	76.66	a	106.1	d	0.2061	bc	0.07378	a	393.8	b	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	LRWC (%)		Plant height(cm)		Chlorophyll a mg.g FW		Chlorophyll b mg.g FW		Leaf area(cm²)		Proline µmol.gFw	
Sc580*0	79.89	a	118.6	ab	0.3280	a	0.09789	a	483.5	a	1.605	a
Ns640*0	77.69	a	117.8	ab	0.2701	ab	0.07822	a	447.0	a	0.9136	b
Sc704*0	79.05	a	122.0	ab	0.2537	ab	0.07678	a	480.0	a	0.9914	ab
Sc580*1	76.07	a	116.4	ab	0.1663	b	0.05778	a	446.2	a	□1.04	b
Ns640*1	76.55	a	124.4	a	0.2468	ab	0.08856	a	478.1	a	0.9802	b
Sc704*1	78.70	a	114.8	ab	0.2501	ab	0.07978	a	444.9	a	1.055	ab
Sc580*2	76.84	a	121.2	ab	0.2182	ab	0.06122	a	425.5	a	1.384	ab
Ns640*2	76.58	a	116.6	ab	0.2127	ab	0.1077	a	465.3	a	1.171	ab
Sc704*2	79.20	a	109.0	b	0.2247	ab	0.08267	a	406.9	a	0.5793	c

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

Table 5. Correlation coefficients between traits

	Leaf area	LRWC	Chlorophyll a	Chlorophyll b	Proline
Plant height	0.708**	0.197	0.412**	0.138	0.072
Leaf area	1	0.281*	0.429**	0.335**	0.197
LRWC		1	0.132	-0.173	-0.021
Chlorophyll a			1	0.459**	-0.094
Chlorophyll b				1	0.143

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

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