

African Journal of Agricultural Economics and Rural Development ISSN: 2375-0693 Vol. 9 (6), pp. 001-004, June, 2021. Available online at www.internationalscholarsjournals.org © International Scholars Journals

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Full Length Research Paper

# Effects of foliar application of salicylic acid on vegetative growth of maize under saline conditions

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### Accepted 21 September, 2020

The study was carried out to evaluate the effects of foliar application of salicylic acid (SA) on vegetative growth of maize under saline conditions at Rafsanjan, Iran in 2009. A factorial experiment based on a completely randomized design with three replicates was used. Treatments consisted four salinity levels (0, 4, 8 and 12 dS/m) and three salicylic acid concentrations (0, 100 and 200 ppm). Traits such as shoot dry weight, stem length, number of leaves, leaf area and chlorophyll were measured. Results of analysis of variance showed that salinity levels and SA concentrations affected shoot dry weight, stem length, number of leaves, leaf area and chlorophyll content significantly (P<0.01). By increasing salinity to 12 dS/m, the earlier mentioned traits decreased to 79.96, 61.2, 25.6, 73.31 and 20.42% respectively. Mean comparisons indicated that plant vegetative growth traits were improved by increasing SA concentration up to 200 ppm. As a result, these traits increased by 84.64, 44.65, 28.20, 74.94 and 38.66% respectively. Salinity and SA interaction had no significant effect on shoot dry weight, stem length, number of leaves, leaf area and chlorophyll of plants.

Key words: Corn, salicylic acid, salinity, vegetative growth.

# INTRODUCTION

Salinity is one of the environmental factors limiting soil fertility and plant production in arid and semiarid regions (Hasegaqawa et al., 2000; Misra et al., 1990, 1997, 2001, 2006; Munns, 2002). This is attributed to the fact that Na<sup>+</sup> competes with K<sup>+</sup> for binding sites essential for cellular function and the latter implication of these two macronutrients in salinity is thought to be one of the factors responsible for the reduction of the biomass and yield components of plants (Hasegawa et al., 2000; Misra et al., 1990, 2001, 2006; Munns, 2002). Main cause of salinityinduced effects on growth and development of plants is accumulation of ions in soil solution and ultimately their absorption in plant cells (Hasegawa et al., 2000). Saltspecific effects on plants are accelerated over time and by the extent of ions accumulation, which eventually rise to toxic level and impose an additional stress on physiological and biochemical processes in plant cells (Munns, 2002; Misra et al., 1990, 2002). This

implies that salt-induced osmotic effects limit the growth, predominantly through power of ion compartmentalization and energy cost in plant cells (Hasegawa et al., 2000). Under stress conditions, such as salinity, drought, low and high temperature, plants produce reactive oxygen species (free radical), which are harmful to plant growth and productivity. Reactive oxygen species deteriorate membrane function, limit NO<sub>3</sub><sup>-</sup> uptake, damage membrane lipids, proteins and nucleic acids under saline conditions (Misra et al., 1990, 2002). Abd-El Baki et al. (2000) reported that nitrate reductase (NR) activity and NR-mRNA were both reduced by salt stress in maize seedlings. Salicylic acid (SA) plays an important role in abiotic stress tolerance, and more interests have been focused on SA due to its ability to induce a protective effect on plants under adverse environmental conditions. Salicylic acid may affect directly on specific enzymes function or may activate the genes responsible for protective mechanisms (Hayat and Ahmad, 2007; Horvath et al., 2007). Salicylic acid controls salinity tolerance in wheat (Shakirova and Bezrukova, 1997; Sakhabutdinova et al., 2003), osmotic stress (Singh and Usha, 2003), mineral nutrition and oxidative stress (Gunes

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Table 1. Analysis of variance (ANOVA) for stem dry weight, stem length, number of leaves per plant, leaf area and chlorophyll contents of maize plants under different salinity levels and salicylic acid treatments.

Sources of variation		Mean of squares					
	df	Stem dry weight (g)	Stem length (cm)	No. of leaves / plant	Leaf area (cm <sup>2</sup> )	Chlorophyll	
Salinity (S)	3	0.231	60.500	2.259	14158.3	93.87	
Salicilic acid (SA)	2	0.053	26.340	2.861	4353.35	277.69	
S*SA	6	0.002 <sup>ns</sup>	0.868 <sup>ns</sup>	0.120 <sup>ns</sup>	108.23 <sup>ns</sup>	9.65 <sup>ns</sup>	
error	24	0.002	0.854	0.389	125.872	5.040	

ns, \* and \*\* means non significant and significant at 5% (P<0.05) and 1% (P<0.01) probability levels, respectively.

Table 2. Mean comparisons of maize plants tested for stem dry weight, stem length, number of leaves per plant, leaf area and chlorophyll contents under different salinity levels.

Salinity (ds/m)	Measured traits					
	Stem dry weight (g)	Stem length (cm)	No. of leaves/ plant	Leaf area (cm <sup>2</sup> )	Chlorophyll	
Control(0)	0.451 <sup>a</sup>	11.17 <sup>a</sup>	4.33 <sup>a</sup>	123.56 <sup>a</sup>	34.55 <sup>a</sup>	
4	0.154 <sup>0</sup>	8.33 <sup>0</sup>	3.33 <sup>b</sup>	50.46 <sup>b</sup>	28.37 <sup>b</sup>	
8	0.169 <sup>b</sup>	7.89 <sup>b</sup>	3.55 <sup>b</sup>	57.07 <sup>b</sup>	28.64 <sup>b</sup>	
12	0.090 <sup>c</sup>	4.83 <sup>c</sup>	3.22 <sup>0</sup>	32.97 <sup>c</sup>	27.5 <sup>0</sup>	

Means with different letter(s) in each column are statistically different based on Duncan multiple range test.

et al., 2007). Salicylic acid appears as a signal molecule or chemical messenger and its role in defense mechanism has been well established in plants (Klessing and Malamy 1994; Gunes et al. 2007). Shruti and Singh (2009) showed that salt-induced deleterious effects in maize seedlings were significantly eliminated by the pretreatment of SA. It is concluded that 0.5 mM salicylic acid improves the adaptability of maize plants to NaCl stress. Gunes et al. (2007) reported that SA could be used as a potential growth regulator to improve plant salinity tolerance. The objective of this study was to investigate the effect of SA on mitigation of deleterious effects of salinity in maize plants.

### MATERIALS AND METHODS

The experiment was carried out in greenhouse to study the effects of salicylic acid (SA) foliar spraying on the vegetative growth of maize (single cross 704) under saline conditions at Valiasr University of Rafsanjan, Iran in 2009. A factorial experiment based on completely randomized design with three replicates was employed. Maize seeds were sterilized by 10% sodium hypochlorite, rinsed with distilled water 5 times and then sown in plastic pots (with depth of 5 to 7 cm) in May 2009. The soil was sandy-loam with pH of 7.4. To prevent salt accumulation in pots, two holes (1 cm in diameter) were created at the bottom of pots for drainage. Pots were filled with sand up to 5 cm height and the rest was filled with the above mentioned soil. The EC of water collected from drainage was used for measuring accumulated salinity of pot soil during the growth. Treatments were a combination of four saline levels (0, 4, 8 and 12 dS.m<sup>-1</sup>) and three salicylic acid

concentrations (0, 100 and 200 ppm). Salinity levels were provided using different but known amounts of NaCl. After emergence, plants were thinned and only three plants were left in each pot. At four leaf growth stage, plants were watered with salt solutions of known EC. Foliar application of SA was done when plants were exposed to salinity for a week and it was repeated two weeks later. Leaf chlorophyll was measured by SPAD (Minolta, Japan). At harvest, the length and dry weight of stem, number of leaves and leaf area were measured. Analysis of variance was applied to determine the effect of salinity levels and SA treatments on the plant growth traits. Means comparison was also used to group the effects of various concentrations of salts and SA.

# **RESULTS AND DISCUSSION**

Salinity and SA independently affected shoot dry weight (Table 1). With increasing salinity to 12 dS.m<sup>-1</sup>, shoot dry weight decreased by 80% compared to the control (Table 2). Shoot dry weight increased with increasing SA concentration. It can be concluded that SA motivates the plant productivity and among the SA treatments, 200 ppm effectively increased shoot dry weight by 84%. According to Shakirova et al. (2003) the positive effect of SA on growth can be due to its influence on the other plant hormones. SA altered the auxin, cytokinin and ABA balances in wheat and increased the growth under normal condition and improved the growth and salinity tolerance under saline condition. Salinity also affected stem length negatively and the highest concentration of salt prohibited stem length by 57% of the control,

SA concentration	on Measured traits							
(ppm)	Stem dry weight (g)	Stem length (cm)	No. of leaves / plant	Leaf area (cm <sup>2</sup> )	Chlorophyll content			
0	0.156 <sup>C</sup>	6.62 <sup>C</sup>	3.25 <sup>b</sup>	49.72 <sup>C</sup>	24.87 <sup>C</sup>			
100	0.205 <sup>b</sup>	7.96 <sup>b</sup>	3.42 <sup>b</sup>	61.49 <sup>b</sup>	29.93 <sup>0</sup>			
200	0.287 <sup>a</sup>	9.58 <sup>a</sup>	4.17 <sup>a</sup>	86.98 <sup>a</sup>	34.49 <sup>a</sup>			

 Table 3. Mean comparisons of maize plants tested for stem dry weight, stem length, number of leaves per plant, leaf area and chlorophyll contents under different SA concentration.

Means with different letters in each column are statistically different based on Duncan Multiple Range Test.

whereas SA seemed to enhance metabolic activities of the cells, which resulted in stem elongation (Table 3). Salinity had a negative effect on the number of leaves (25.6% reduction), while SA increased this trait but its effects did not show the same amount of positive reaction (only 28.20%) compared to other traits. Leaf area was also affected significantly by salinity (Table 1) and the highest concentration of salinity (12 dS.m<sup>-1</sup>) restricted leaf area and reduced it to one forth of the control. High concentration of SA (200 ppm) caused an increase of 74.94% in leaf area (Table 3). Zhou et al. (1999) also indicate that SA increases the leaf area in sugarcane plants, which is consistent with our results. Chlorophyll content was adversely affected by increasing salt concentration and about 20.42% reduction appeared at 12dS.m<sup>-1</sup> salinity (Table 2). Such a result was obtained by Misra et al. (2006) who reported 71% reduction in chlorophyll content of Phaseolus seedlings exposed to NaCl. Salinity treatment is known to affect photosynthetic pigments due to the retardation of their synthesis or the induction of degradation during salt stress (Misra et al., 1997). Yamane et al. (2004) reported the reduction in chlorophyll by salt stress and concluded that these results induced injury in chloroplasts is dependant on light and that H<sub>2</sub>O<sub>2</sub> and OH are responsible for the deleterious effects of salt stress on chlorophyll content. In turn, the highest amount of SA application was accompanied with 38.66% increase in chlorophyll concentration in the leaves. The metabolic effects of SA depends on the plant type, the amount and the application method of that, and wheat 10<sup>-5</sup>M SA increased the amount in of photosynthetic pigments while the higher concentrations decreased it (Hayat and Ahmad, 2007). Considering the results, it could be concluded that salinity generally provides a slow growth and development of cells. especially in the leaves which is confirmed by Munns (2002) who stated salinity reduces plant growth through lessening or stopping the leaf expansion. This factor suppresses the turgor pressure and metabolic activities in the cells that are observed as low number and small size of leaves associated with short plant height. Additionally, salinity disturbs mineral nutrient absorption and ion balance in the plant organelles (Misra et al., 1990). Therefore, a decrease in leaf and stem length could also be attributed to mineral nutrient deficiency in the root regions or environmental

factors like salinity which produce toxicity in the minerals being absorbed by roots. It is evident that chlorophyll, as a photosynthetic unit, exists in the cell chloroplast and is capable of capturing light energy and converting it to chemical energy. According to the harmful chemical reactions created by salinity in the plants (Misra et al., 2001), photosynthetic efficiency may be decreased in response to low nutrient uptake or poisonous metabolites entrance into the cell (Misra et al., 1990, 2002). Therefore, salinity not only markedly lowered the total photosynthetic efficiency in the plant through insufficient leaf number and leaf, but also caused low enzymatic reactions for the synthesis of photosynthetic materials through low chlorophyll concentration of leaves. Total dry weight of plants was remarkably limited by increasing salinity, indicating that the whole energy chain reactions use substrates to neutralize toxic effects penetrated into plant cells. These results also presented an interrelation between dry weight index, leaf area and chlorophyll concentration in plants.

A positive reaction of SA on plant growth regulations may be associated with chemical changes in plants through preventing poisonous ions like Na<sup>+</sup> and Cl<sup>-</sup> and enhancing easy uptakes of ions like NO<sub>3</sub><sup>-</sup>, Mg<sup>2+</sup>, Fe<sup>2+</sup>, Mn<sup>2+</sup> and Cu<sup>2+</sup>. The same results about positive effects of SA on corn growth were found by Hussein et al. (2007).

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