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Effects Of Salicylic Acid On Growth, Yield And Chemical Contents Of Pepper (*Capsicum Annuum* L) Plants Grown Under Salt Stress Conditions

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ABSTRACT: Experiments were carried out to investigate the effect of salinity stress on growth, chemical constituents and yield, and to examine whether salinity stress can be offset by the exogenous application of salicylic acid (SA) on sweet pepper (Capsicum annuum L. cv. Orlando). Salinity stress (2000, 4000 or 6000 ppm) decreased plant growth and marketable yield but SA (250 ppm) treatment as foliar spray counteracted significantly the harmful effects of low and moderate salinity stress levels (2000 and 4000 ppm) and partially counteracted the harmful effects under the highest salinity stress level (6000 ppm). Moderate salinity stress increased chlorophyll content, soluble sugars, proline and free amino acids. The highest level of salt stress decreased the activities of catalase (CA), peroxidase (POX), super oxide dismutase (SOD) and polyphenol oxidase (PPO) enzymes. While SA improved all chemical concentrations and enzyle activities. These results provide support for the field application of salicylic acid to alleviate the harmful effects of salinity on pepper plants.
Key words: Pepper, salinity stress, salicylic acid, growth, enzyme activity.

INTRODUCTION

Pepper (*Capsicum annuum* L.) is an important agricultural crop, not only because of its economic importance, but also for the nutritional value of its fruits, it is an excellent source of natural colors and antioxidant compounds important for human health (Howard et al., 2000). Pepper is a moderately sensitive to salt stress (Lee, 2006), and it is grown under protected glasshouse conditions in temperate regions and in the open field under warm Mediterranean climates. Where it is grown in Saudi Arabian soils, it is frequently exposed to saline conditions brought about by saline irrigation water containing amounts of salts including sodium chloride (Kijne, 2003).

Salinity imposes stress conditions on crop plants (Hajer et al., 2006) and affect growth and chemical contents and has been shown to limit pepper yield (Paridam and Das, 2005). Salt stress severely inhibits plant growth for two reasons: first by an osmotic or water- deficit effect of salinity and second by a salt-specific or ion-excess effect of NaCl. Moreover, plants subject to salinity stress conditions produce cytotoxic activated oxygen that can seriously disrupt normal metabolism, through oxidative damage of lipids, proteins, and nucleic acids (Abbaspour, 2012). To defend against such oxidants, plants have evolved specific protective mechanisms, involving antioxidant molecules and enzymes that protect against the potentially-cytotoxic species of activated oxygen. Adaptation to salt stress requires alterations in gene expression and also the application of exogenous substances such as salicylic acid.

Salicylic acid (SA) is a signaling or messenger molecule in plants and induces plant tolerance against various biotic and abiotic stresses (Horvath et al., 2007). SA also plays an important role in the regulation of some physiological processes in plants such as effects on growth and development, ion uptake and transport and membrane permeability (Simaei et al., 2012). Exogenous SA alters the activities of antioxidant enzymes and increases plant tolerance to abiotic stress by decreasing generation of ROS. It has been found that SA has different effects on stress adaptation and damage development of plants that depend on plant species, concentration, method and time of SA application (Metwally et al., 2003). Furthermore, SA is a potential non-enzymatic antioxidant and an important signal molecule for modifying plant responses to environmental stressors. Some earlier reports display that exogenous SA can ameliorate the impairing effects of drought stress in different species (Arfan et al., 2007). SA has obtained particular attention because of inducing protective effects on plants under NaCl salinity (Simaei et al., 2011). Several studies have shown that the effects of cytotoxicity induced by salt stress can be ameliorated by the exogenous application of SA (Simaei et

al., 2012). If such amelioration can be sustained then such treatments offer the opportunity for in-field protection against this stress.

The objectives of this work were to study the effect of salinity stress on growth, yield and endogenous bio-constituents of sweet pepper (Capsicum annuum L.) plants, and to examine whether the harmful effects of salinity stress can be offset by the exogenous application of SA.

MATERIALS AND METHODS

Pot experiments were conducted in under greenhouse conditions during summer seasons of 2010/2011, to investigate the effect of salt stress on growth, yield and chemical constituents of sweet pepper and to find out if SA can counteract the harmful effect of salinity on plants. Sweet pepper seeds were obtained from authorized agricultural company and sown in trays. Forty five days after sowing (6-7 leaves), seedlings were transplanted into pots (30 cm inner diameter) containing 8 kg of air-dried sandy soil, two plants/pot. Plants were fertilized with Sangeral complete fertilizer (20%N : 20%P : 20% K plus essential micro nutrients) in two equal portions; the first one was added during the seedling stage and the second was added at the beginning of flowering stage. Two weeks after transplanting, irrigation solutions containing one of four levels sodium chloride (00, 2000, 4000 or 6000 ppm) were used gradually (in increments of 2000 ppm each day) to prevent osmotic shock. Irrigation solutions were supplied according to plant need and to maintain a slight reserve of water in the pot saucer. Foliar application of salicylic acid SA (250 ppm) was done twice, 21and 28 days after transplanting. The experiment consisted of 8 treatments (4 salt treatments with or without SA) and was arranged in a completely randomized design with 6 replicates for each treatment to make a sum of 48 pots. Sixty days after completion of salt treatments, the following measurements were recorded:

Growth characters

Plant height (cm); number of leaves/plant; leaf area (cm^2/plant) and shoot dry weight (g) were measured.

Chemical parameters

Total chlorophyll was measured spectrophotometrically in acetone extracts according to Wettstien et al. (1957).

Total soluble sugars was determined colorimetrically at absorbance wavelength of 490 nm using sulfuric acid-phenol method as described by Duboi et al. (1956)

Proline colorimetric determination proceeded according to Bates et al. (1973) based on proline's reaction with ninhydrin. For proline colorimetric determinations, a 1:1:1solution of proline, ninhydrin acid and glacial acetic acid was incubated at 100ºC for 1 hour. The reaction was arrested in an iced bath and the cromophore was extracted with 4 ml toluene and its absorbance at 520 nm was determined.

Total free amino acids was measured spectrophotometerically in 80% ethanol extracts using ninhydrin method as described by Troll and Lindsley (1955).

Enzyme activities

Catalase activity was assayed by adding 0.1 ml of plant extracts to 1.4 ml of freshly prepared 13.2 mM H202 in 0.05 M K2HPO4 (pH 7.0). The solution was mixed, and a loss of absorbance was determined at 240 nm. Units of catalase were calculated by using a molar absorbance index for H202 of 43.6 as described by Pine et al. (1984).

Peroxidase was determined by using o-dianisidine (Worthington Biochemical Corp., 1972). To 1.5 ml of 0.01 M phosphate buffer (pH 6.0), 0.01 ml of o-dianisidine (1% in methanol) was added and mixed; 0.1 ml of enzyme extract was added and mixed, then 0.1 ml of 0.3% freshly prepared H202; the solutions were mixed, and the change of absorbance was recorded at 460 nm for 3 to 5 min. Units of peroxidase were calculated by using a molar absorbance of 11.3 x 103.

SOD was determined by the procedure of Marklund and Marklund (1974). The relative SOD activities of fractions eluted was measured spectrophotometrically through the reduction of nitroblue tetrazolium in the presence of light and riboflavin.

PPO was measured calorimeterically according to Smith and Stotz (1949). A volume of 5.0 ml. of 0.1 M phosphate-citrate buffer of pH 6.0, 5.0 ml. of 0.6 M leuco dye (1.0 PM), 1.0 ml. of 0.1 M catechol, and finally 1.0 ml. of enzyme extract were all well mixed at 30OC. The absorbance was quickly measured calorimeterically.

Yield and its components

Fruit setting percentage was determined as the number of fruits set per number of flowers produced.

Two fruit picking were taken from each treatment at 90 and 120 days from transplanting, total yield was calculated (summation of the two pickings).

For each treatment, number of fruits/plant; fresh weight of fruits/plant (g) and dry weight of fruits/ plant (g) were recorded.

Statistical analysis

The collected data were analyzed statistically using analysis of variance according to Snedecor and Cochran (1989) with the aid of COSTAT computer program. Treatment means were compared using the least significant differences (LSD) at 5% level.

RESULTS AND DISCUSSION

Vegetative growth

All growth characters of sweet pepper plants were decreased with increasing salinity stress levels with the greatest reduction observed at the highest salinity level (Table 1). It was clear that the most negative effect of salt stress was observed on the reduction of number of leaves and leaf area per plant. At salt concentration of 2000 ppm, the reductions of plant height, no. of leaves/plant, LA/plant and dry weight of shoots were 25%, 26%, 28% and 22%, respectively. While at 4000 ppm these reductions were 27%, 29%, 29% and 40%, respectively. The corresponding reductions at 6000 ppm of salt were 40%, 42%, 58% and 70%.

Data in the same table indicate that SA treatment improved the growth characters at all levels of salt stress and also control plants, it is therefore acting as growth stimulants. It seems that SA treatment could completely counteract the negative effect of low (4000 ppm) and medium (2000 ppm) salt stress and partially counteract the harmful effect of medium and high (6000 ppm) salt stress.

Table 1. Effects of salinity stress and salicylic acid on growth parameters of pepper plants.

Salt (ppm)	SA trt.	Plant height (cm)	No. of leaves /plant	Leaf area (cm2)/plant	Shoot d.wt (g)
00	- SA	40.3b	45.5b	1425b	11.2b
	+ SA	48.4a	60.2a	1815a	17.5a
2000	- SA	33.2c	32.2d	1022c	8.7c
	+ SA	44.2b	51.6b	1645a	13.5b
4000	- SA	28.5d	28.2d	876c	6.3c
	+ SA	35.6c	39.8c	1015c	8.2c
6000	- SA	20.7e	24.5d	546d	3.4d
	+ SA	22.6e	26.2d	575d	4.2d

Numbers in a column followed by the same letter are not significantly different at 0.05 level of significance.

The inhibitory effects of salinity on growth of pepper plant reported in this study (Table 1) were typical of the effects of high soil salt availability and are probably due to decreased water absorption and disturbed metabolic processes leading to decreased meristematic activity and/or cell enlargement (Kaydan and Okut, 2007) coupled with an increase in respiration rate resulting from higher energy requirements. Hussein et al. (2012) reported that there are two ways that salinity could retard growth, by damaging growth cells so that they cannot perform their functions or by limiting their supply of essential metabolites. Salinity stress is known to retard plant growth through its influence on several vital factors of plant metabolism, including osmotic adjustment (Sakr and El-Metwally, 2009) nutrient uptake, protein and nucleic acid synthesis, photosynthesis (Zaibunnisa, et al., 2002), organic solute accumulation, enzyme activity, hormonal balance and reduced water availability at the cell level all of which result in reduced plant growth and ultimately reduced yield. Furthermore, increased salt content in the irrigation water may cause direct and indirect effects on leaf water relations and stomatal closure which influence CO2 ex-change and photosynthetic rate. Increased salt content in irrigation water may be directly toxic to plants, which in turn, lowered carbohydrate accumulation in the plants (Morales et al., 2008).

Simaei et al. 2011) attributed the depressing effects of salt stress on plant growth to an increase in reactive oxygen species which play an important role in damaging all classes of biologically important macromolecules including DNA and the generation of H_2O_2 and lipid hydro-peroxides which cause membrane changes. Reductions in fruit yield are largely attributable to decreases in the viability of pollen or the receptivity of the stigmatic surface (Sakr et al., 2004) and substantially increased abscission of flowers or young fruit due to ethylene induction by salinity. Other factors affecting cell division and cell expansion, such as tissue water status and the concentration of certain plant hormones e.g. ABA are also involved in the regulation fruit set under stress.

Chemical contents

There was insignificant increase in total chlorophyll, soluble sugars and proline content under salt stress treatment except for the highest level of salt which decreased all mentioned parameters (Table 2). Moreover, all salt treatments decreased the content of free amino acids in plant leaves, as compared with unstressed plants. On the other side, SA treatment increased significantly total chlorophyll, soluble sugar content, proline and free amino acids content as compared with SA untreated stressed-plants. The data show that the applied SA completely counteracted the harmful effects of salinity stress.

The increased total chlorophyll under low levels of salinity recorded in this study is in agreement with the finding of Hussein et al. (2012) on pepper plants and Liu et al. (2007) on Aeluropus littoralis plants who found that salt stress increased Chl a and Chl b contents, but the Chl a/Chl b ratio declined, which implies the stimulation of Chl a accepted from NaCl was smaller than that of Chl b. This increase may be attributed to the thickness of the leaves under salt stress rather than to stimulation of pigment formation.

Table 2. Effects of salinity stress and salicylic acid on chemical parameters of pepper plants.

Salt (ppm)	SA trt.	Total Chl. (mg/g f.wt)	Soluble sugars (mg/g dwt)	Proline (mg/g dwt)	Free AA (mg/100g)
00	- SA	3.2b	65.2d	3.2c	68.5ab
	+ SA	4.2a	115.5b	2.8	95.6a
2000	- SA	3.9a	74.5cd	4.5b	56.4c
	+ SA	4.6a	142.4a	2.5c	82.4a
4000	- SA	3.7ab	88.6c	5.8a	50.2c
	+ SA	5.2a	156.8a	2.0c	77.5a
6000	- SA	1.2d	60.d	6.2a	42.5d
	+ SA	2.0c	87.3c	1.5d	60.6b

Numbers in a column followed by the same letter are not significantly different at 0.05 level of significance.

With increasing salinity levels, total chlorophyll in pepper leaves significantly decreased (Table 2), this reduction may be related to enhanced activity of the chlorophyll-degrading enzyme, chlorophyllase, as suggested by Mishra and Sharma (1994) who indicated that increasing saline increased oxidation of chlorophyll leading to its decreased concentration. Earlier studies reported that the reduction in leaf chlorophyll content of the plants grown in NaCl stress has been attributed to the destruc-tion of chlorophyll pigments and instability of the pig-ment protein complex. Furthermore, increased salt content also interferes with protein synthesis and influ-ences the structural component of chlorophyll (Jalee et al., 2008).

As recorded in Table (2), salinity stress levels and SA treatment both increased proline, soluble sugars and free amino acid contents in pepper leaves during the growing season. Several functions are proposed for the accumulation of these compounds in plant tissues submitted to stress including osmotic adjustment, stabilization of proteins and membranes, being a scavenger of free radicals, improvement of the stability of some cytoplasmic and mitochondrial enzymes, and increased protection of proteins and enzymes or membranes (Sakr et al., 2007; Simaei et al. 2011). Previous studies showed that soluble sugar increased in oat plants with NaCl increasing (El-Tayeb, 2005). With salicylic acid, the leaves fill up more soluble sugar and proline (Szepesi, 2006). The increasing of photosynthesis carbohydrate is a signal for water deficiency tolerance. The high carbohydrate concentration with its role to reduce water potential helps to prevent oxidative losses and protein structure maintenance during water shortage. Also carbohydrates play a molecule role for sugar responsible genes that give different physiological response like defensive response and cellular expansion (Simaei et al. 2011).

There are many reports about increasing and decreasing of protein level in salinity stress. The soluble protein and free amino acid in barley increased with NaCl increasing. The study of maize plant and also all amino acids increased with salicylic acid (El Tayeb, 2005; Hussein et al., 2007). The increasing of amino acid in the plant tissue under stress is related to protein fraction. The cause of protein reduction at high salinity stress condition may be du to the prevention of nitrate reductase activity and to protein synthesis reduction (Hussein et al., 2007).

Enzyme activity

Data in Figs. (1 and 2) showed that activities of CAT, POX, SOD and PPO enzymes increased insignificantly with increasing salt stress until 4000 ppm, above which the activities of all enzymes were decreased as compared with control plants. SA treatment showed a linear increase in enzyme activities in salt stressed and unstressed plants until 4000 ppm. The increase in enzyme activity at 6000 ppm stressed plants, in the presence of SA, was insignificant for CAT, POX and PPO and significant for SOD, as compared with SA-untreated plants under the same level of salt stress.

To mitigate and repair damage initiated by reactive oxygen, plants have developed a complex antioxidant system. The primary components of this system include some enzymes such as catalase (CA),

POX

peroxidase (POX) superoxidase dismutase (SOD) and polyphenol oxidase (PPO). Many components of these antioxidant defence system can be found in different sub-cellular compartments (Vaidyanathan et al., 2003). These antioxidant enzymes and metabolites are reported to increase under various environmental stresses (Sakr et al., 2013) and this increase was also evident in the current work.

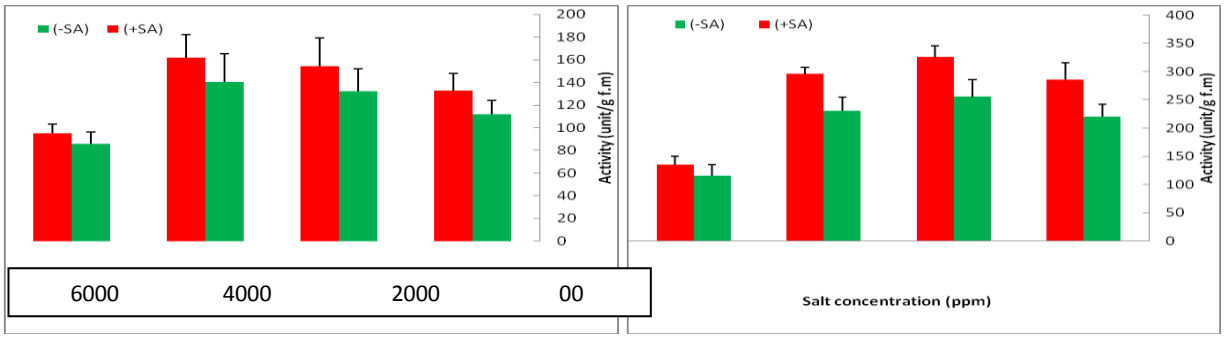

Figure1. Effects of salt stress and salicylic acid on the activity of catalase (CA) and peroxidase (POX) in pepper plants. (vertical lines represent the SE)

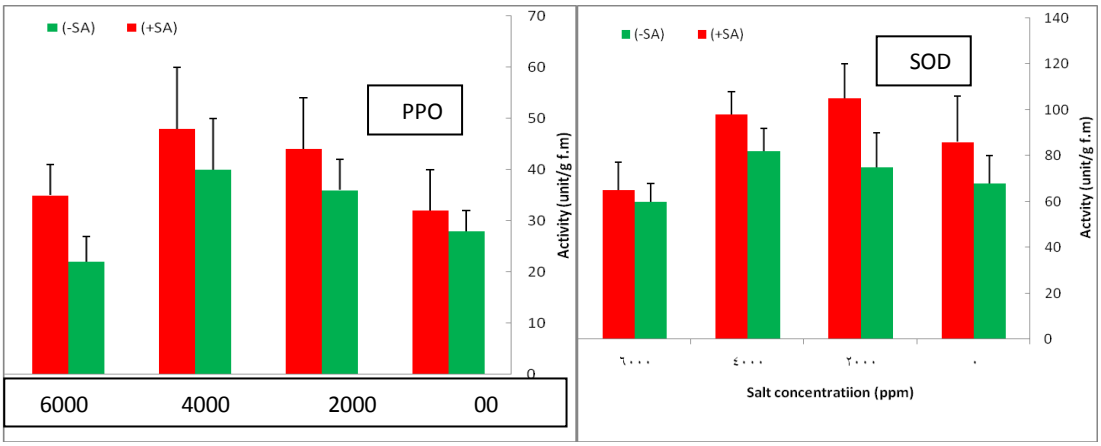

Figure2. Effects of salt stress and salicylic acid on the activity of Dismutase (SOD) and phenol oxidase (PPO) in pepper plants (vertical lines represent the SE)

Salinity stress levels clearly affected enzyme activity in pepper shoots. Superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) have all been reported to exhibit different changes in plants at 10^{-7}M SA or 10^{-4}M SA (Sakhabutdinova et al., 2003). Senaratna et al. (2000), reported that SA and APX confer tolerance to pepper plants and the tolerance was associated with changes in antioxidants such as glutathione reductase, dehydroascorbate reductase and monode-hydroascorbate reductase. SA treatment also increased the level of reduced glutathione (GSH) with an increase in the ratio of reduced to oxidised glutathione (GSSG) indicating higher antioxidant potential (Sakr et al., 2013). Proline is one of the important components of the adaptation of plants to salinity (Abbaspour, 2012) and pretreatment with SA also contributed to accumulation of this amino acid under stress possibly through maintaining an enhanced level of ABA in the plants (Ervin, 2005).

Yield and yield components

Fruit setting, total fruit yield and fresh and dry weights of pepper fruit decreased with increasing level of salinity stress (Table 3). The high salt stress (6000 ppm) reduced fruit set%, number of fruits/plant, fresh weight of fruits/plant and dry weight of fruits /plant by about 60%, 73%, 75% and 66%, respectively, as compared to control plants. On the other side, applied SA increased fruit setting, fruit yield, and fresh and dry weights of pepper fruits compared to salt-stressed plants without SA treatment. These increases were observed at all levels of salinity stress including control plants. It seems that SA counteracted the negative effects of salt stress.

Table 3. Effects of salinity stress and salicylic acid on yield components of pepper plants.

Salt (ppm)	SA trt.	Fruit set (%)	Total No. of fruits/plant	F wt. of fruits/plant	D wt. of fruits/plant
00	- SA	12.5	10.4	355	22.5
	+ SA	21.2	14.2	596	38.8
2000	- SA	10.8	6.3	220	15.5
	+ SA	17.3	10.6	412	28.4
4000	- SA	8.4	4.7	157	10.2
	+ SA	12.2	7.5	215	20.3
6000	- SA	5.5	2.8	87	7.6
	+ SA	7.2	3.6	110	9.7

Numbers in a column followed by the same letter are not significantly different at 0.05 level of significance.

It was obvious that increasing salinity decreased economic of fruit yield due to the decreased number of perfect flowers and fruit set and imperfect fruit production and this has been reported elsewhere (Grattan et al., 2002). Furthermore a reduction in leaf area reported in this study (Table 1) might result in reduction in the supply of carbon assimilate due to a decrease in the net photosynthetic rate and biomass accumulation (Sakr et al., 2007). According to the data recorded in this investigation, it was shown that salinity stress decreased many parameters including leaf number, leaf area, accumulation of dry matter, photosynthetic pigments, and sugar content all of which will ultimately decrease pepper yield. While SA treatment alleviate the harmful effect of salt stress on yield components and improved the yield parameters due to its positive effect on growth parameters as shown in Table (1).

CONCLUSION

Salt stress even at moderate levels significantly decreased growth and biomass production of pepper plants. High salinity stress decreased the photosynthetic pigments, sugar content and the yield of pepper. The adverse effects of salt stress on the growth and productivity of pepper can be mitigated by foliar spray of SA. However, effectiveness of SA in alleviating the adverse effects of salinity stress was salt-dose dependent. The results presented in this investigation clearly indicate that it is possible to ameliorate the effects of salinity by the exogenous application of salicylic acid (SA), a compound known to up-regulate the plants natural defense against salt stress. The implications of this work are that it may be possible to develop field applied protection against salt stress.

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