Physiological and Biochemical Effects of Salicylic Acid Pretreatment Seeds in *Pisum sativum* L. Cultivated under Natural Saline Field Condition

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Abstract: The effects of seed pretreatment with Salicylic acid (SA) on certain physiological aspects, growth and productivity of peas under natural saline condition were evaluated. The data indicated that seed pretreatment with 10^{-4} M SA increased the activity of glutamate dehydrogenase (GDH) by about 1.5 times in pea leaves associated with higher accumulation of proline and water content in different organs compared with the control. In addition, number of secondary roots, xylem vessels/root, root nodules /plant and the nitrogen active zone area achieved doubled increase in response to SA treatment. Free amino acids and protein contents in the leaf, nodule and seeds were also increased by about 108% in the SA-treated seeds. Moreover, SA treatment improved the amount of globulin, prolamin and glutelin whereas decreased albumin content in pea seeds. Furthermore, levels of enzymatic and nonenzymatic antioxidants, such as free phenols, peroxidase (POX) and catalase (CAT) activity were increased by approximately 2-3 times in leaf, root nodules and seeds. Seed yield of pea was increased in SA treated plants by 400 kg per hectare compared with the control. It could be concluded that SA may regulate the biosynthesis of nitrogenous compounds in pea plant under natural saline environments.

Keywords: peas, salicylic acid, nodule anatomy, antioxidants, glutamate dehydrogenase.

INTRODUCTION

Pea (*pisum sativum,* L.) is considered an important winter vegetable crop for Egypt and other countries, mainly for fresh or dry pulses rich in protein, carbohydrates, fibers, vit.B1 and antioxidants content (Mukerji, 2004). Pea is sensitive to salinity as all legumes and degree of salinity level affects rate of yield losses (Maas, 1990). Seed yield of peas decreased by about 13.3% per unit increase in soil salinity after 0.6 dS m^{-1} which is the threshold value of peas (Duzdemir *et al.,* 2009). Injurious effect of salinity on crops was attributed to decline in water uptake or inactivate enzymes and disturbance of solutes (Greenway and Munns, 1980;Munns and Tester, 2008).The negative effect of salt stress on nitrogen fixation in legumes have been widely reported (Zahran, 1992). Salinity stress causes oxidative stress by inducing the generation of reactive oxygen species (ROS) in plants, such as H_2O_2 and O_2^- and oxidative damage can be minimized by antioxidant defenses that scavenge or prevent the generation of ROS (Ashraf and Harris, 2004). Gill and Tuteja (2010) cited that plants possess very efficient enzymatic (superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, monodehydro-ascorbate reductase, dehydroascorbate reductase, glutathione peroxidase, guaicol peroxidase, and glutathione-S- transferase, and non-enzymatic (ascorbic acid, glutathione, phenolic compounds, alkaloids, non-protein amino acids and α-tocopherols) antioxidant defense systems.

Salicylic acid (SA) is an endogenous plant growth regulator, plays miscellaneous physiological roles in plants and potentially alleviates the disturbing effect generated by various biotic and abiotic stresses (Hyate *et al.,* 2010). However, a lot of literatures indicate that exogenous application of salicylic acid to the stressed plants can potentially alleviate the toxic effect of salinity such as, wheat (Hamada and Al-Hakimi, 2001), tomato (Tari *et al.,* 2004), barley (El Tayeb, 2005), Brassica juncea (Yusuf, *et al.,* 2008), pepper (Elwan and Elhamahmy, 2009). SA also plays a major role during the early stages of Rhizobium-legume symbiosis. Nodule number, N_2 fixation and protein content were either negatively affected by SA application in *Vicia sativa*, *pisum sativum* or positively in *Phaseouls vulgaris*, *Lotus japonicus* and *Glycin max*, as mentioned by Van Spronsen *et al.* (2003). Pretreatment with salicylic acid activated various antioxidant enzymes in maize (Janda *et al.,* 2000) and banana (Kang *et al.,* 2003) exposed to chilling stress.

Exogenous application of SA was found to affect the activities of the enzymes of nitrogen metabolism in different legume crops. Glutamate dehydrogenase (GDH; EC 1.4.1.2), a mitochondrial, stress responsive enzyme, activate the oxidation of glutamate. Catabolic role for glutamate dehydrogenase implies an important regulatory function in carbon and nitrogen metabolism. (Yamaya *et al.,* 1984; Robinson *et al.,* 1991). Glutamic acid participate in proline biosynthesis in plant. Proline is used as an enzymic protector that contributes in macromolecules structure and is main source of energy and nitrogen to confront salinity (Munns and Tester, 2008).

Therefore, the aim of this study was to investigate the effect of pretreatment of pea seeds with different concentrations of SA on GDH activity and other chemical compounds such as proline and amino acids and their relation with growth and productivity in pea grown under natural saline soil.

MATERIAL AND METHODS

Plant material, treatments and growth condition:

The experiment was conducted at the natural saline in Experimental Farm Faculty of Agriculture, Suez

Canal University, Ismailia, Egypt, (latitude, 30° 36' N; longitude,32°14' E; altitude, 10m above sea level) during 2008 season. The physical and chemical properties (Chapman and Pratt, 1961) of the soil used are presented in Table 1.

Seeds of pea (*Pisum sativum*, L. cv. Master B) were provided from the Egyptian Vegetable Research Center, Ministry of Agriculture, Egypt. They were soaked in distilled water as control, or in 10^{-2},10^{-4} or 10^{-6} M salicylic acid dissolved in dimethylsulfoxide (100 seeds in 100 ml) for 12 h, after surface sterilization with 1% sodium hypochlorite (3min.), sowing at 15th of September was took place in hills, 10 cm apart, on one side of ridges, 65 cm width. During cultivation season, the average day/night temperature was 24/16, 21/10, and 16/8 in November, December and January, respectively (http://arabic.wunderground.com). Plots were distributed according to blocks experimental design with three replicates. One week after cultivation, the plants were inoculated with 1 ml per seedling of 10^8 cells ml^{-1} *Rhizobium leguminosarum bv. Viciae* from an exponential culture in peptone-yeast extract medium (Beringer, 1974). Plants were irrigated and fertilized as normal agricultural practices.

Measurements and observations:

Plant biomass and water content:

Shoot, root systems and nodules fresh weights of 10 plants from each replicate were determined using gravimetric method after 50 days from sowing. Water content % of shoot, root systems and nodules were calculated at 90 °C and drying at 70 °C up to constant weight.

Pod yield: Marketable pods were once harvested at the end of experiment (100 days after sowing) then average weight of pods and seed/plant as well as seed and pod yield per hectare were recorded.

Determination of total free phenols, free amino acids, proline and ascorbic acid: Ethanolic extract (96% ETOH) of leaves, nodules and seeds were prepared according to Abdel-Rahman *et al.* (1975), free phenols were determined spectrophotometrically (Beckman DK-2 Spectrophotometer) at 650nm with Folin–Ciocalteu reagent according to AOAC (1996). Total free amino acids concentration was estimated using the method of Rosen (1957) with ninhydrin reagent spectrophotometricaly at 650 nm. Free prolin was assayed in fresh plant material according to Bates *et al.* (1978). L-proline was used as a standard. Ascorbic acid concentration (mg/g F.W.) in the seed was estimated according to Pearson (1970).

Preparation of enzyme extracts of nodules, seeds and leaves: 0.5 g fresh nodules, leaves or seeds was homogenized by using a mortar and pestle with 0.1M phosphate buffer (pH 6.5) at 4 °C and stirred for 20 min. The suspension obtained was filtered through one layer of muslin cloth and then centrifuged at 18,000×g for 15 min, 4 °C. The supernatant was used to determine activity of enzymes (Urbanek *et al.*, 1991) as follows:

Peroxidase (POX; EC 1.11.1.7) assay: The reaction mixture consisted of 3.5mL of 0.1M phosphate buffer (pH 6.5), 0.3mL of 0.1% o-dianisidine solution, 0.2mL enzyme extract and 0.2mL of 0.2M hydrogen peroxide solution (Urbanek *et al.*, 1991). The reaction mixture was incubated at 30 °C for 10 min and oxidation of o-dianisidine was measured by changes in optical density at 430nm (BeckmanDK-2Spectrophotometer). Corrections were done for the oxidation rate of o-dianisidine in the absence of H_2O_2 in the reaction mixture. The activity of POX was expressed as optical density per milligram of protein per minute. One unit of POX activity (AU) was taken as the change of 1.0 unit of optical density per minute.

Catalase (CAT; EC 1.11.1.6) assay: The reaction mixture consisted of 0.01mL enzyme extract and 2.99mL hydrogen peroxide-phosphate buffer (pH 6.8) prepared after dilution of 0.16mL of H_2O_2 (10%, w/v) to 100mL phosphate buffer (Urbanek *et al.*, 1991). The oxidation of H_2O_2 was measured by changes in optical density at 240nm in 30 s intervals for 5min (Beckman DK-2 Spectrophotometer). The unit of CAT activity was defined as the amount of enzyme, which decomposes 1mmol H_2O_2 per minute at 25 °C.

Glutamate dehydrogenase (GDH; EC 1.4.1.2) assay: The reaction mixture consisted of 0.2 ml α-ketoglutarate 0.2M, 2.3 ml Tris-HCl buffer solution 0.1 M (pH 8), 0.1 NADH 5mM and 0.2 ml enzyme extract. After 5 min. at 30 °C, optical density at 340 nm was measured after the add ion of 0.2 ml $(NH_4)_2SO_4$ 1.5M in 30 s intervals for 5min (Beckman DK-2 Spectrophotometer). The activity of GDH was expressed as optical density per milligram of protein per minute. One unit of GDH activity was defined as the amount needed to oxidize 1.0 µmol of NADH per min at 3O °C. (Loulakakis and Roubelakis-Angelakis, 1990).

Protein fractionation in the seeds: According to A.A.C.C. (2003), the sequential extraction of the proteins was carried out. Each extraction step was performed in two stages at room temperature with ratio of 30 mL of solvent/g of seed. All solvents contained 0.1 mM phenylmethylsulfonylfluoride, a protease inhibitor, to prevent proteolysis. Between the stages, the extraction residue was separated by centrifugation at 10 000 g for 20 min at 4°C and precipitated by adjusting to pH 3.0 with 2 N HCl. Albumin was extracted with water. Globulins were extracted from the albumin-free pellet with 0.1 M NaCl, 0.01 M K_2HPO_4 (pH 7.5), 0.001 M EDTA. Prolamin was exteracted with 70% aqueous 2-propanol. Glutelin was extracted with 0.1 M NaOH; 0.1 M $Na_2B_4O_7$ (pH 10) and 0.1 M $Na_2B_4O_7$ + 1% of SDS (pH 10). The protein content in the supernatants was measured by the method of Bradford (1976) using bovine albumin serum (BSA) as a standard.

Anatomical studies:

Nodules specimens located on third secondary root were killed and fixed in F.A.A., then dehydrated in ethyl alcohol series, embedded in Paraffin wax, sectioned to thickness of 15 mm, double stained with Safranin and Light green, cleared in Xylene and mounted in Canada balsam according to Willey (1971). Measurements were calculated by eyepiece micrometer.

Statistical analyses:

All data were statistically analyzed as randomized

complete blocks design (Steel *et al.,* 1997); using the MSTAT-C statistical package (M-STAT, 1990) and means were separated by LSD test, $P \leq 0.05$.

RESULTS

GDH activity and proline content:

Assay of GDH activity showed an induction due to SA pretreatments, especially in the leaves and nodules. 10^{-4} or 10^{-6} M SA elevated GDH activity by 0.5 fold in nodule and seed or 1.5 fold in leaf of pea plant under the natural saline condition (Fig.1). Higher activity of GDH in different organs of plant under SA treatments clears that, SA up-regulated GDH in the present investigation. In the same trend, seed soaking with SA significantly ($P \leq 0.05$) increased proline accumulation in pea different plant organs. Elevation of SA concentrations indicated increase in free proline content in leaf, root and nodules. However, the level of free proline was much higher, 67.9 and 22.77 µmol/g FW in treated leaf and root with 10^{-4} M SA, respectively and 11.77 µmol/g FW in nodules of treated seed with 10^{-6} M SA (Fig. 2).

The antioxidants level:

Pretreatment pea seeds with SA induced the synthesis of different antioxidants in different parts of pea plant grown in soil with high salt concentration (Table 2). High free phenols content, POX and CAT activities in root nodules, leaf and seeds of pea plants were observed with 10^{-4} or 10^{-6} M SA treatments, and their values increased by approximately 2-3 times compared to control. Vitamin C in the form of ascorbic acid was also non-significantly increased in seeds at all concentrations.

The anatomical characters of root nodules:

The significant highest values of maximum width and length of effective nitrogen fixing active zone in cross section of root nodules (1281.2 and 3225.4 µm, respectively) was recorded in plants treated with SA at low concentration (10^{-4} M) compared to control and other SA concentrations. Also, treatment with SA may have stimulated the presence of doubled nodule or doubled active zone in the same nodule as shown in Fig. 3 and Table 3. In addition, treated seeds with SA in all concentrations showed double number of xylem vessels /root compared to control ones, reached to approximately 35 vessel/root compared to 19 vessel/root in control. The increase in number of xylem vessels/root may be led to an increase in the transportation capacity of water under saline condition. Lowest significant values of thickness of periderm (18.2 µm) was recorded in plants seed treated with 10^{-6} M SA, which be led to formation of many active zone in the same nodule as shown in Fig.3. Increasing the area of active nitrogen fixation zone and formation of many active zones in the same nodule directly increased the amount of nitrogen which was fixed biologically.

Free amino acids, protein contents and protein fractions:

Table (4) showed that, pretreatment of pea seeds with different concentrations of SA increased free amino acids content in nodule, leaf and seeds. Moreover, free amino acids in nodule, leaf and seeds were also increased by (18, 29 and 35 %) as affected by 10^{-4} M SA treatment. This increment was associated with an enhancement of protein content in all examined parts of plant. SA at 10^{-4} M also improved the content of protein by about 59, 25 and 108% in nodule, leaf and seeds, respectively as shown in Table (4).

Pretreatment with SA altered the ratio of different protein types in pea seeds. Albumin content was decreased in all SA treatments, especially with 10^{-6} M SA by about 6.5% compared to control. In contrast, SA pretreatment with different concentrations enhanced the content of other protein types. 10^{-4} SA resulted in the highest contents of globulin, prolamin and glutelin in seeds (34%, 24% & 24%) respectively, compared to control (Table 4).

FW, water content and pod yield:

Seed treatment with low concentration of SA showed high FW values of shoot, root and root nodules compared with than those treated with high concentrations of SA or control. 10^{-4} M SA significantly increased FW of all previous organs by 23, 620 and 216 % respectively, (Table 5). The same table showed that, seeds treated with SA at all concentrations were able to retain more water in their tissues than control ones. SA at 10^{-4} M increased water content of shoot and root by 3.5, 8.2%, respectively, whereas decreased water content of root nodules by 5.1% as compared to control. On the other hand, number of secondary roots & root nodules/plant (Fig.4,Tab.5) was increased with SA treatment at all concentrations, especially at 10^{-4} M, reach to maximum number, 22 secondary root and 42 nodules per plant compared to 11 secondary roots and 13 nodules in control. Data in Table (5) showed also that, pea plants negatively or positively responded to the pretreatment of SA according to the concentration used. In general, SA at 10^{-4} M enlarged the average pods and seed weight(g/plant), average pods and seed weight(Ton/hectare) by approximately (40g, 10g) per plant, (2, 0.4ton) per hectare, respectively as compared to the control under the present investigation.

DISCUSSION

Annual addition of chemical fertilizers to the soil increased the concentration of salts, which negatively affect the growth and production of pea plant. Pretreatments of seeds with different growth regulators such as indole acetic acid, gibberline, cytokinin or SA alleviated the negative effect of stress in many aspects of plant growth and development (Hayat *et al.,* 2010). In the present work the EC of cultivated soil (Table 1) of peas was 3.5 ds/m, classified as moderately saline soil. Salt stress led to the generation of reactive oxygen species which oxidize important chemical compounds in plant cell, especially photosynthetic pigments. In addition, nucleic acids were blocked and proteins, then enzymes were inhibited (Munns and Tester 2008). SA pretreatment was found to positively affect pea seeds germination rate and improve the seedling performance, under experimental conditions (unpublished data).

Pretreatment of pea seeds with SA at low concentration doubled the number of secondary roots/ plant and xylem vessels/root compared to control (Table

3, 5; Fig. 3, 4). Subsequently, increasing of water uptake under the present investigation. This increment of water content led to enhance the FW of shoot, root and root nodules. The increase of FW of these organs may be the result of high water absorption under saline conditions. FW was increased by 2, 6 folds in root nodules and root, respectively (Table 5). Similar results were reported by Sandoval-Yepiz (2004) who found that, SA plays an important role in the bioproductivity of plants that could be linked to the observed effect of promoting root length of plants. Also, Elwan and El-Hamahmy (2009) reported that SA application at low concentration (10^{-6} M) positively increased the foliage fresh and dry weights, fruit number, average fruit weight, fruit yield, vitamin C and carotenoids content of pepper plants grown in greenhouse under saline soil. Also, Zhou *et al.* (1999) found that, SA can reduce the injurious effect of salinity by increasing photosynthesis rate in wheat under saline conditions. SA or acetyl salicylic acid enhanced the germination percentage of carrot seeds (Rajasekaran *et al.,* 2002) and *Capsicum annum* (Korkmaz, 2005) at low temperatures.

Plant grown under saline condition suffered from water stress, as a result of low water potential of soil. Adaptation of plant to salinity started with excluding Na^+ into cell vacuole by Na^+/H^+ antiporter located in tonoplast and synthesis of different osmolytes into cytoplasm in order to maintain water molecules around the essential macromolecules such as protein, enzymes and nucleic acids to prevent their denaturation (Rai, 2002). Therefore, biosynthesis of high levels of proline from glutamic acid in leaves was occurred, and then translocated to the rest of plant organs, especially roots and root nodules. Proline may be one of the main osmolytes in peas, maintains high water content in the examined plant parts, which increased FW and productivity of peas under saline conditions (Fig. 2, Table 5). GDH plays an important role in proline biosynthesis from glutamic acid. Results demonstrated that, SA may up-regulate the activity of GDH in different parts of plants such as, root nodules and seeds by 0.5 time and leaves by 1.5 times (Fig. 1). This increment was associated with higher level of proline accumulation in different examined plant organs (Fig.2). Subsequently, the increase of proline in plant tissues improved maintaining of water content in different organs under salinity. Eraslan *et al.,* 2007 found that, SA application regulated the proline accumulation and decreased the toxic ion (Cl, B) accumulation, both in shoot and storage root of carrot grown under combined stress of salinity and boron toxicity.

Plant grown under salt stress posse's very active antioxidants system to eliminate any ROS in different parts of plant. Antioxidants found in high concentrations in root nodules, due to nitrogenase activity, were reduced with raising level of oxygen in nitrogen active zone. Quenching of O_2 occurred with biosynthesis of Leghemoglobin or antioxidants like phenols, ascorbic acid, peroxidase, catalase (Najafi *et al.,* 2007). SA treatment increased the antioxidants levels by approximatly 2-3 times (Table 2) in different organs (root nodules, leaves and seeds). These results were in agreement with observations indicated that, SA enhanced the activities of antioxidant enzymes, CAT, POX and superoxide dismutase(SOD), when sprayed exogenously to the drought stressed plants of *L. esculentum* (Hayat *et al.,* 2008) or to the salinity stressed plants of *B. juncea* (Yusuf *et al.,* 2008) and *pisum sativum* (Zahran and Ashraf 2009). Also, David *et al.* (1998) demonstrated that cells immediately peripheral to the infected region of peas root nodules contain elevated levels of Ascorbate peroxidase that scavenges H_2O_2 and thus, prevents oxidative damage in N_2-fixing legume root nodules.

Increase of root nodules/plant, length, width of nitrogen fixing zone as well as presence of many active zones in the same nodule (Tab.3 and Fig 3), as a result of SA application; directly enhanced the amount of nitrogen which was biologically fixed. Duplication the number of secondary roots and root nodules/plant by SA treatment, clear that SA may regulate the division and elongation of plant cell through its regulation of I.A.A and cytokinin levels in plant, (Shakirova *et al.,* 2003). Moreover, SA may induce differentiation of xylem vessels of pea roots initiated from cambial cells. SA as a simple phenol participated in lignin biosynthesis. Systemic acquired resistance appears to result from increased levels of certain defense compounds as SA and lignin (Shulaev *et al.,* 1997). By contrast, Van Spronsen *et al.* (2003) demonstrated that, exogenous application of 10^{-3}M SA delayed nodule formation and decreased the number of nodules from roots of pea plant. Moreover, Farrukh *et al.* (2008) found that high number of nodules was formed in *pisum sativum* at 0.0 and 1.0 dsm^{-1}, whereas at higher salinity levels, no nodules were formed. Increasing the thickness of periderm tissue around the root nodule can prevent division of nodule cells. Anatomical behaviors of root nodules verified that SA affects the subsequent nodule development, in contrary to other observations (Van Spronsen *et al.,* 2003) who mentioned that SA at low concentration decreased the growth and development of nodules in *pisum sativum*,L.

NH^{4+} in the single free form is very toxic to plant cells (Addiscott and Benjamin, 2004). Therefore, plant induced different enzymes like glutamate synthetase to quench all NH^{4+} molecules into stable, non toxic form, such as amino acids. Free NO^{3-} or NH^{4+} in plant tissues are considered very hazard molecules to animals and humans, causing different kinds of cancer. For safety, plant tissues must have NO^{3-} or NH^{4+} ions at very low concentrations (Addiscott and Benjamin, 2004). Accumulation of free amino acids was enhanced in root nodules, leaves and seeds (Table 4). Free amino acids were increased by 35% in pea seeds. Subsequently, protein content was increased also, especially in seeds by 108% under 10^{-4} M SA pretreatment. Results indicated that, SA participate in ammonium translocation to amino acids and that improved the quality of peas for animal and humans.

The quality of agricultural products is very important to consumer, such as higher content of protein and antioxidants in pea seeds. Pea proteins can be divided into water-soluble albumins, salt-soluble globulins, alcohol-soluble prolamines and acid/alkali-soluble glutelins. The main storage proteins of pea seeds

are albumin while globulins, prolamines and glutelins are detected in small amounts (Mandal and Mandal, 2000; Martinez-Villaluenga *et al.,* 2008). Amount of albumin, globulin, prolamin and glutelin in pea seeds was also up-regulated under SA treatment with salt conditions. Globulin was increased by 34% and both of prolamin and glutelin were increased by 24%, compared to control (Table 4). In this connection, Kumar *et al.,* (1999) cited that, content of total protein was increased in seed of soybean sprayed with SA, and this increase might be due to enhanced activity of nitrate reductase, enzyme responsible for nitrate reduction into ammonium.

Finally, accumulation of the plant biomass, proline, free amino acids and different antioxidants compounds positively guide to increase pod yield or seed/hectare, by 2 ton and 400 kg per plant, respectively (Table 5). These results are also agreed with Sandoval-Yepiz (2004) as well as Elwan and Elhamahmy (2009).

Table (1): Physical and chemical properties of the experimental soil.

Mechanical analysis (%)			Chemical analysis		Cations (mM)				Anions (mM)		
Sand	Clay	Silt	P^H	EC (ds/m)	Ca^{2+}	Mg^{2+}	K^+	Na^+	HCO_3^-	Cl^-	$SO4^{2-}$
91	3	6	8.27	3.5	10.44	4.9	0.88	3.25	6	7.28	10.76

Table (2): The antioxidants content in different organs of peas in response to SA pretreatments.

SA/M	free phenols mg/g DW			VC mg/g Fresh Seed	units/mg protein/min					
					POX activity			CAT activity		
	nodules	leaf	seed		nodules	leaf	seed	nodules	leaf	seed
0.0	9.4^b	33.2^c	12.6^b	41.1^a	0.97^d	1.28^c	1.17^b	2.26^c	2.72^d	1.29^d
10^{-2}	9.9^b	42.8^b	20.7^a	42.4^a	1.36^c	3.8^b	2.27^a	2.83^b	4.21^c	1.59^c
10^{-4}	18.2^a	67.4^a	20.8^a	43.2^a	1.98^a	4.65^a	2.46^a	5.37^a	7.23^a	2.41^a
10^{-6}	18.7^a	67.5^a	20.4^a	41.3^a	1.8^b	4.35^a	2.11^a	5.23^a	5.3^b	2.21^b

Values are the means of 10 plants per replicate. Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple range tests.

Table (3): The anatomical characters of secondary root and nodule of peas as affected by different concentrations of SA:

SA/ M	Presence of		Maximum width of active zone (µm)	Maximum length of active zone (µm)	Thickness of periderm (µm)	Number of xylem vessels
	Doubled nodule	Doubled active zone				
0.0	-	-	576.1^d	1386.7^d	45.3^b	19^c
10^{-2}	+	-	979.7^c	1606.2^c	68.2^a	35^b
10^{-4}	+	+	1281.2^a	3225.4^a	30.4^c	38^a
10^{-6}	+	+	1053.3^b	2764.6^b	18.2^d	38^a

Values are the means of 10 plants per replicate. Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple range tests.

Table (4): Impact of SA on, free amino acids, protein content and protein fractions in different organs of pea:

SA/ M	free amino acids content mg/g DW			Protein content mg/g DW			Seed Protein Fractions mg /g seed			
	nodule	leaf	seed	nodule	leave	seed	Albumin	Globulin	prolamin	Glutelin
0.0	79.2^c	136.3^c	156.3^b	126.1^c	315.2^b	327.1^d	379.7^{ab}	33.8^c	62.4^c	20.7^c
10^{-2}	79.7^c	143.7^b	157.6^b	188.2^a	322.9^b	386.5^c	395.2^a	42.1^b	70.6^b	24.8^{ab}
10^{-4}	97.9^a	165.7^a	191.6^a	185.5^a	340.6^a	435.3^a	362.5^b	51.9^a	82.7^a	27.5^a
10^{-6}	91.4^b	145.1^b	188.1^a	166.7^b	345.3	413.1^b	354.9^b	31.5^c	53.4^d	22.1^{bc}

Values are the means of 10 plants per replicate. Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple range tests.

Table (5): Effect of different concentrations of SA on some growth parameters and yield of pea

SA/ M	FW(g)/plant			Water content %			number/plant		Yield (g)/plant		Yield (Ton)/hectare	
	Shoot	Root	Nodule	Shoot	Root	Nodule	Secondary roots	Root nodules	pod	seed	pod	seed
0.0	73.7^b	2.7^d	1.02^d	88.3^c	86.4^c	87.5^a	11^c	13^d	144.2^b	48.3^b	6.8^b	2.4^{ab}
10^{-2}	77.4^b	4.93^c	1.78^c	90.0^b	91.5^b	87.3^a	16^b	24^c	127.4^c	40.5^c	6.4^c	2.1^b
10^{-4}	97.5^a	8.9^a	3.18^a	91.8^a	94.6^a	82.4^c	22^a	42^a	183.3^a	59.7^a	8.7^a	2.8^a
10^{-6}	65.8^c	7.57^b	2.36^b	91.7^a	91.7^b	85.3^b	14^{bc}	31^b	144.2^b	48.6^b	7.1^b	1.6^c

Values are the means of 10 plants per replicate. Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple range tests.

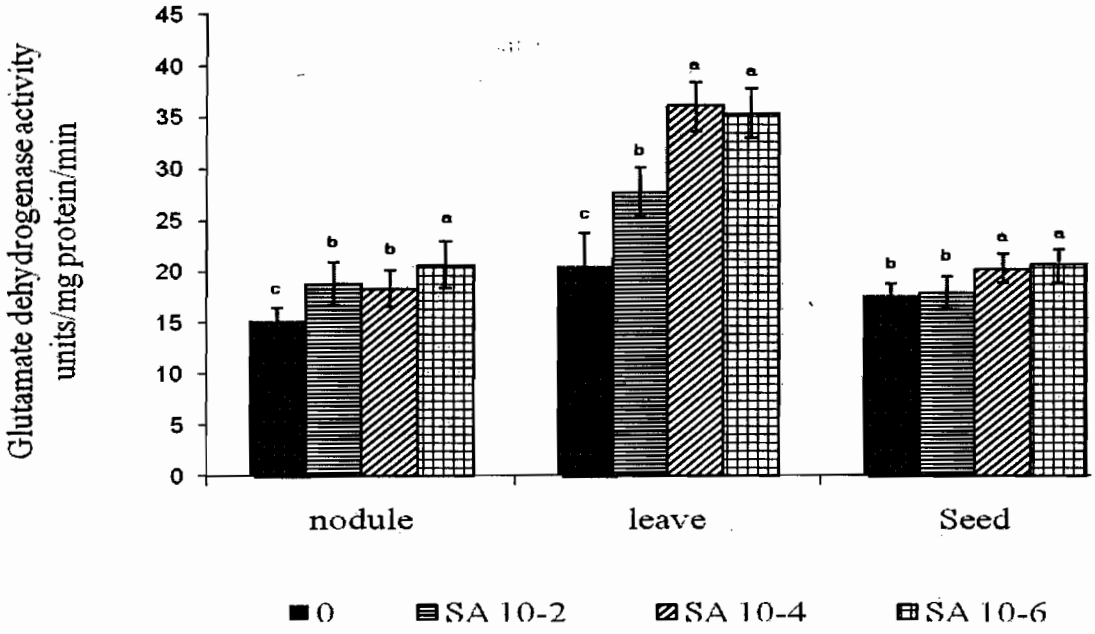

Fig. (1): Glutamate dehydrogenase (GDH) activity as affected by different concentrations of SA. (means±SE, n = 4). Means denoted by different letters are significantly differentat P≤0.05 as determined by Duncan's test

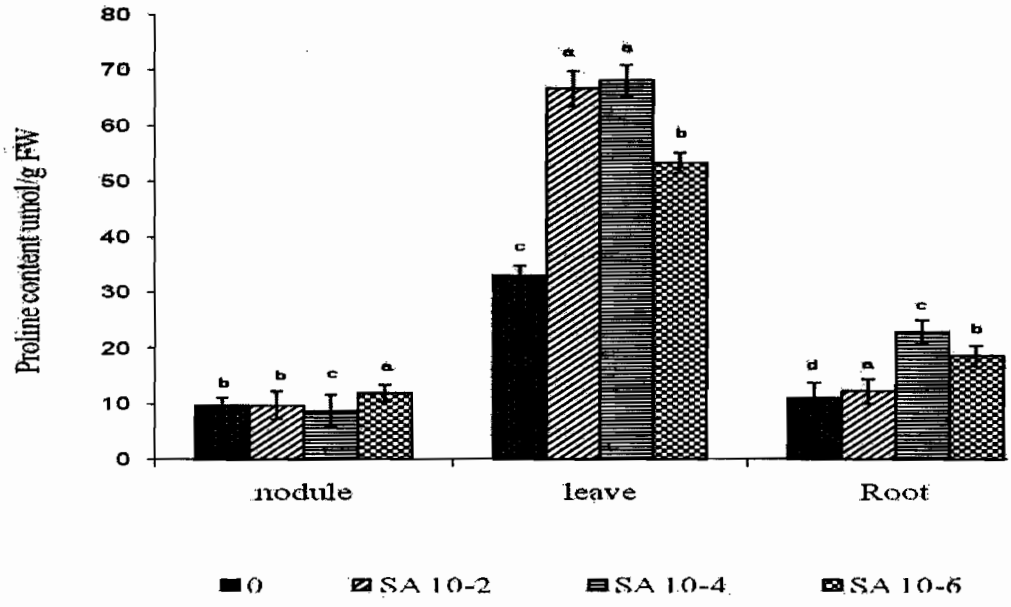

Fig. (2): Proline level in different organs of pea in response to SA application. (means±SE, n = 4). Means denoted by different letters are significantly differentat P≤0.05 as determined by Duncan's test

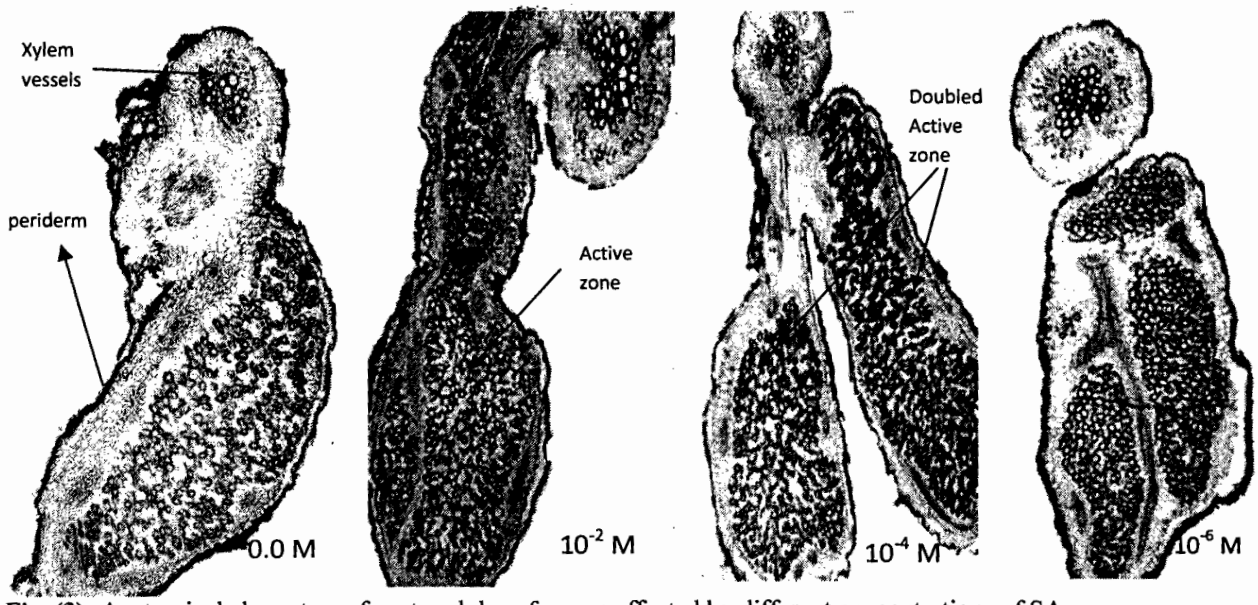

Fig. (3): Anatomical characters of root nodules of pea as affected by different concentrations of SA.

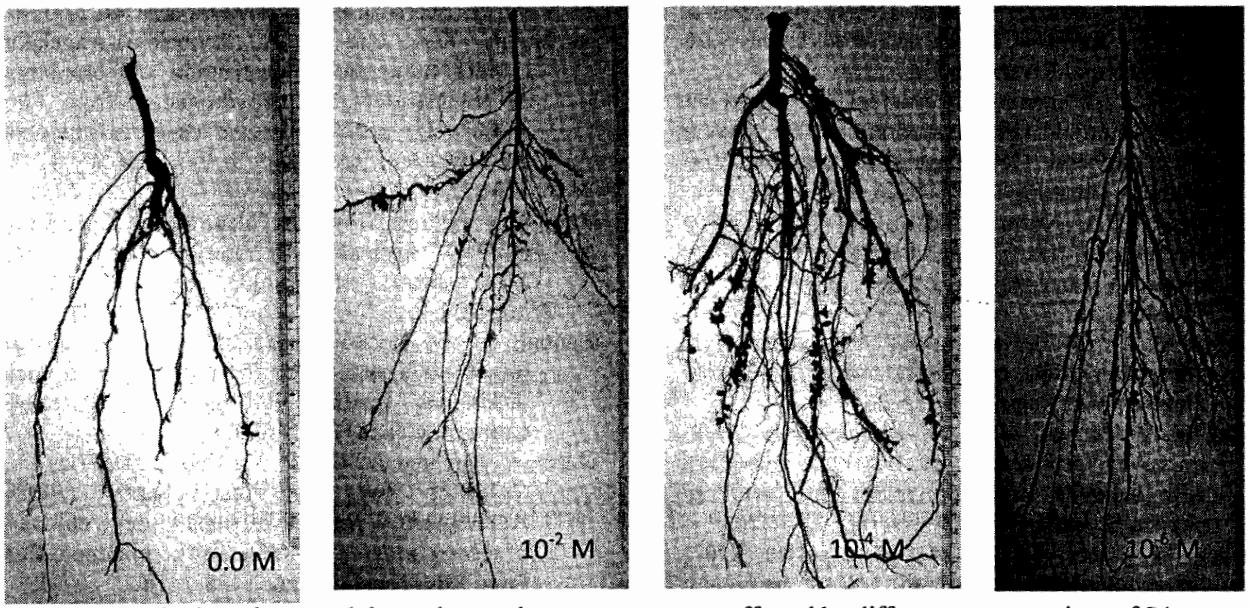

Fig. (4): Distribution of root nodules and secondary roots on pea as affected by different concentrations of SA.

CONCLUSION

Balance of biosynthesis and degradation of nitrogenous compounds may adapt plants to survive and produce under different types of stress. Peas accumulates nitrogen in the form of amino acids or protein in their leaves and seeds. In the same time, degradation of amino acids must be done to synthesis different kinds of osmolytes to face the injurious effects of stress. To explain this difficulty pretreatment of pea seeds in different low concentrations of SA before sowing in natural saline soil (3.5 ds/m) was examined. SA was increased many growth parameters, such as shoot, root and root nodules FW in order to maintain high water content, due to accumulation of high levels of proline in these parts associated with enhancing glutamate dehydrogenase activity, responsible for biosynthesis of proline from glutamic acid in different parts of plant. Also, SA application in low concentration induced biosynthesis of some antioxidants, phenols, vitamin C, peroxidase and catalase in leaf, root nodules and seed of pea plant. Treatment with SA was doubled root nodules number/plant and the active zone in the same nodule, may be increased N_2 fixation led to increase free amino acids and protein content in leaf, nodule and seed. SA also enhanced Globulin, Prolamin and Glutelin; while decreased Albumin content in pea seeds. Finally, all previous parameters increased pod and seed yield of peas by 2 and 0.4 ton per hectares, respectively.

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التاثيرات الفسيولوجية والكيموحيوية لمعاملة البذور ما قبل الزراعة بحمض السالسيلك لنبات البسلة تحت ظروف ملوحة الحقل الطبيعية

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فى هذه الدراسة تم تقييم تأثير معاملة البذور قبل الزراعة بحمض السالسيلك على الصفات الفسيولوجية والنمو وانتاجية نبات البسلة النامي تحت ظروف ملوحة الحقل الطبيعية. اظهرت النتائج ان معاملة البذور بحمض السالسيلك بتركيز 10^{-4} مول ادى لزيادة نشاط انزيم الجلوتامات ديهيدروجينيز بمقدار ١,٥ مرة فى اوراق النباتات والذى ارتبط بتراكم كميات عالية من البرولين وزيادة المحتوى المائى للاعضاء النباتية المختلفة مقارنة بالكنترول. بالاضافة الى تضاعف عدد الجذور الجانبية وعدد اوعية الخشب وعدد العقد الجذرية لكل نبات وكذلك مساحة المنطقة النشطة فى تثبيت النيتروجين لكل عقدة جذرية نتيجة الاستجابة لحمض السالسيلك والتى ادت مباشرة الى زيادة كمية الاحماض الامينية الحرة والبروتين فى الاوراق والعقد الجذرية والبذور. ازداد تركيز البروتين بنسبة ١٠٨ % فى البذور مقارنة بالكنترول. من ناحية اخرى ادت المعاملة بحمض السالسيلك الى زيادة كمية جلوبيولين وبرولامين وجلوتيلين البذرة فى حين انخفض البيومين البذرة. كما ازداد نشاط مضادات الاكسدة مثل الفينولات الحرة والبيروكسيديز والكتاليز بمقدار من ٢-٣ مرات فى كلا من العقده الجذرية والاوراق والبذور. ازداد محصول البذرة بمقدار ٤٠٠ كجم / هكتار مقارنة بالكنترول. اوضحت الدراسة ان حامض السالسيلك ربما ينظيم تخليق المركبات النيتروجينية فى نبات البسلة تحت ظروف الملوحة الطبيعية.