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Original Paper

Differential responses of esterase isoenzyme of peanut to salinity and drought as influenced by salicylic acid

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Abstract
The effect of salinity, drought and salicylic acid on growth and esterase expression of peanut plants were studied.Seeds of peanut were able to germinate even under relatively high concentration of NaCl (157.5 mM) or PEG (25%). In general, seedling growth was less tolerant to salt stress than vegetative growth stage of peanut plants grown in hydroponic culture containing NaCl, vice versa was detected when PEG was used.While the application of 0.1 mM salicylic acid (SA), inhibit peanut seed germination, it improved seedling and vegetative growth parameters under moderate concentration of NaCl (105 mM). Seedling and vegetative growth parameters were significantly reduced by increase the concentrations of PEG. Application of SA improved growth parameters of peanut plants subjected to 10% PEG.In hydroponic culture, peanut was more sensitive to PEG than NaCl where plants wilted in three days under relatively high PEG concentrations. Root of peanut seedlings increased the esterase expression under the influence of moderate salinity (105 mM) and drought (10% PEG) stresses through increase the number and staining intensity of isoenzyme forms.Increase the number of isoenzyme forms and/or staining intensity as an indicator of increased activity of the enzyme was detected when peanut plants were hydroponically grown in solution with relatively high NaCl or PEG.Application of SA increased the number and/or the staining intensity of isoenzyme forms during seedling or vegetative growth in hydroponic culture containing relatively high concentration of NaCl or PEG.

Key words: NaCl, polyethylene glycol, esterase isoenzyme, salicylic acid, seed germination, *Arachis hypogaea.*

Introduction

Among biotic and abiotic factors, salinity and drought affect negatively on the productivity of agricultural systems which hinders future governmental plans to achieve enough food. Since man cannot stop desertification and soil salinization, they must increase the ability of plants to survive and maintain their economic growth under drought and saline conditions (Ashraf, 2009; Qadir *et al*. 2014).

Polyethylene glycol (PEG 6000) is an inert osmotic agent which cannot penetrate the apoplast, so it results in water withdrawn from the cell and cell wall. Consequently, PEG was used as an available and cheap factor to mimic drought under field conditions (Verslues *et al*., 2006). Availability of low water is the main aspect under the influence of both drought and salinity. In addition to osmotic stress, plants suffer from ion toxicity of both Na^+ and Cl^- under salinity stress. To ensure plant survival, plants develop specific mechanisms to control the negative effects of osmoticum and ion toxicity (Mahajan and Tuteja, 2005), the key action of these mechanisms is modulation of gene expression.

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Well seed germination and seedling growth should be established as guarantee for continuous vegetative growth leading to commercial yield (Karagić *et al*., 2010) but it depended on the suitability of the environmental conditions. On the other hand, un-favorite conditions including salinity or water stress retarded these stages (Kalefetoglu Macar *et al*., 2009). Oil producing plants, such as peanut, expressed a valuable degree of salinity tolerance via accumulation of lipids as osmoregulation (Younis *et al*., 1987; Abdel-Rahman and Hassanein, 1988; Hassanein, 1999). During seed germination, lipid hydrolysis was increased due to the increase ester-hydrolyzing enzymes resulting in free fatty acids from triglycerides for β-oxidation in the glycoxysome (Devlin and Witham, 1986; Younis *et al*., 1987). Esterases are proteins which hydrolyze ester bonds and are present in several isoenzyme forms. Esterases have hydrolytic action on broad range of substrates and they could be used as a molecular marker for somatic embryogenesis and organogenesis (Hassanein *et al*., 1999). Under the influence of biotic and abiotic stresses, variation in esterase expression was registered in peanut (Hassanein, 1999) in barley (Tamás *et al*., 2005) and tomato (Hassanein, 2004).

Previous studies indicated that salicylic acid (SA) was used as a hormone-like substance due to its important role in photosynthesis, stomatal function, transpiration, antioxidation and inhibition of Na^+ and Cl^- accumulations (Gunes *et al*., 2007; Arfan *et al*., 2007; Xu *et al*., 2008). Consequently, it was used to alleviate environmental stress such as salinity and drought (Shakirova *et al*., 2003; Singh and Usha, 2003). On the other side, exogenous application of SA in rooting medium of maize inhibited plant growth (Nemeth *et al*. (2002). While induction of abiotic stress tolerance was not detected by SA in plants, application of SA by pre-soaking of seeds, incorporation in hydroponic culture, in water of irrigation or foliar application improved growth of plants under abiotic stresses (El-Tayeb, 2005; Szepesi *et al*., 2009).

Plants change protein expression during development and/or stress condition. It is regulated before or after transcription (Scandalios, 1974). Variation in gene expression can be detected by studying the expression of isoenzymes and/or SDS PAGEs. In this concern, appearance or disappearance of isoenzyme gives an indication about how gene expression is influenced by specific agent. In addition, change in staining intensity of one or more bands indicates to change in the enzyme activity (Khavkin and Zabordina, 1994; Hassanein *et al*., 1999). Also, this indicates that genes involved in expression of these isoenzyme forms are differentially expressed under the influence of studied biotic or abiotic condition (Chawla, 1991; Hassanein *et al*., 1999; El-Tayeb and Hassanein,2000). Comparative studies on esterase isoenzyme expression under different abiotic stresses in oil producing plants were limited. Then, the objective of this study was to clarify the differential responses of esterase isoenzyme expression of peanut to salinity and drought stresses at different developmental growth stages in the absence or presence of SA. In addition, this work investigated which stage of peanut growth was more sensitive for salt or drought stress: seed germination, seedling or vegetative growth stage.

Materials and methods

Seed germination and seedling growth as influenced by different concentrations of NaCl or PEG:

Peanut seeds (*Arachis hypogaea*cv; cultivar Shandaweel 1) were obtained from Sohag Agricultural Research Center, Sohag, Egypt. Uniform-healthy seeds were germinated in plastic pots (11 cm width x 9 cm height) containing sawdust. Each pot was irrigated with 200 ml of 1/4 strength of Hoagland solution (Hoagland and Arnon, 1950) containing different concentrations of NaCl (0.0, 45, 105 or 157.5 mM) or polyethylene glycol 6000 (0, 5, 10, 15, 20, 25 or 30 %) with or without 0.1 mM salicylic acid (SA). Seeds were germinated under controlled condition (26 ± 2 ºC at 16/8 h photoperiod of 100 µmol m^{-2} s^{-1} irradiance and 70 % relative humidity) for 15 days. Three replicates with five seeds

per each were used for each treatment. Percentage of seed germination, root and shoot lengths, and leaf number/shoot were estimated. Also, fresh and dry masses, and water content of root, stem and leaves were determined. Esterase expression under the influence of different treatments of salinity or drought was analyzed in seedlings roots and shoots.

Hydroponic plant growth as influenced by different concentrations of NaCl or PEG:

Peanut plants obtained from seeds germinated in sawdust irrigated with tap water for ten days were transferred to grow in hydroponic cultures to investigate the effect of different concentrations of NaCl or PEG with or without SA on plant growth. The hydroponic cultures were established by placing plants in conical flasks (250 ml) containing 200 ml of Hoagland solution (1/2 strength) and supplemented with different concentrations of NaCl (0, 45, 105 or 157.5 mM) or PEG (0, 5, 10, 15, 20, 25 or 30 %) with or without 0.1 mM SA. Three replicates with three plants/each were used for each treatment. Conical flasks were wrapped with aluminum foil to prevent the exposure of roots to light. Cultures were incubated under controlled conditions for 15 or three days for salinity or drought, respectively. Cultures were daily aerated for 5 min by using aeration pump. Growth parameters were estimated. Esterase expression under the influence of different treatments of salinity and/or drought was studied in roots, stems and leaves.

Esterase isoenzyme analysis

One gram of plants grown under the influence of different treatments was grounded on ice in 2 ml homogenization buffer (0.1 µM Tris-HCl, pH 7.0 and 2mM cystein). The obtained homogenates were subjected for centrifugation at 15000 rpm for 15 min using cooling centrifuge at 4 ºC. Supernatants were collected using micropipette and transferred for immediate electrophoresis using 7.5% polyacrylamide slab gels. In each well, 100 µl of supernatant of each sample were loaded. Electrophoresis was carried out for 6 h at 10 ºC in run buffer (0.025 M Tris-base + 0.192 M glycine (pH 8.9) with 2 mA per sample. Esterases were detected following staining method described by Brewer (1970).

Statistical analysis

Data were presented as means ± standard deviation (SD) as described by Snedecor and Cochran (1980). Analysis of variance (ANOVA) was carried out using the software of SPSS 16, the significance level was measured, $P \leq 0.05$ was considered as significant.

Result and discussion

Salinity and drought are two of the major environmental abiotic stresses in Egypt and many other countries, consequently, they were the subject of this study. Seed germination and seedling growth of peanut were decreased under the influence of salt stress (Table 1). The ability of peanut to geminate was 93.3 and 80% under relatively moderate and high salt stress, respectively.This ability of high seed germination of peanut under slat stress can be used to cultivate peanut commercially in saline soil.Seed germination is considered the most important developmental phase in the plant life and it is affected by salinity (Misra and Dwivedi, 2004). On the other side, most of seedling growth parameters such as root and stem lengths, leaves number, and root, stem and leaves fresh masses were significantly decreased under salt stress. Under the influence of highest NaCl concentration, the values of root and shoot fresh masses were reduced by 77.64 and 56 %, respectively. It indicated that salt stress adversely affected root growth more than shoot growth as was reported by Van-De-Vanter (2001). Reduction in root and shoot growth was considered the most important indicator for the response of plants to environmental stresses (Jamil and Rha, 2004).

Under the influence of salt stress, seed germination of peanut was decreased by the application of SA as was reported by Jadhav and Bhamburdekar (2011). On the other side, the enhancing effect of SA on seed germination under slat stress was reported in other plants (McCue *et al*., 2000*;* Shakirova *et al*., 2003). Salicylic acid improved the growth parameters of roots of unstressed and stressed peanut seedlings (Table 1), it may be due to

increase the water content of the plant organs. Under moderate NaCl condition, SA improved the determined seedling growth parameters. Under the effect of the highest NaCl concentration, SA improved slightly the water content of roots and it was associated with increase root growth (length and fresh weight). The increase in fresh and dry matter of salt stressed plants due to the application of SA was related to induction of antioxidants and salt tolerant in plants (Gunes *et al*., 2005).

NaCl Coc. (mM)	Germination percentage (%)	Root length (cm)	Stem length (cm)	No. of leaves/shoot	Root F.W (g)	Stem F.W. (g)	Leaves F.W. (g)	Root W.C. (%)	Stem W.C. (%)	Leaf W.C. (%)
0.0 (Control)	100	9.1 ± 1.35	8 ± 1.37	7.00 ± 0.57	1.61 ± 0.00	0.75 ± 0.03	1.38 ± 0.19	84.95 ± 0.86	83.36 ± 0.29	82.77± 0.58
45	100	5.7* ± 0.17	5.77 ± 0.25	6.00* ± 0.00	1.12 ± 0.10	0.57 ± 0.06	0.69* ± .17	82.72*± 0.97	82.51 ± 1.93	81.84 ± 1.79
105	93.33	5.00* ± 1.00	3.37* ± 0.40	6.00* ± 0.00	0.58* ± 0.14	0.35* ± 0.02	0.35* ± 0.04	83.88 ± 0.84	81.70 ± 1.54	80.74 ± 0.29
157.5	80	3.33* ± 0.75	3.00* ± 0.20	4.00* ± 1.00	0.36* ± 0.10	0.33* ± 0.05	0.24* ± 0.04	81.35* ± 0.36	83.43 ± 0.35	80.51± 1.43
0.0 + SA	100	9.7 ± 1.04	8.70 ± 1.7	7.00 ± 0.00	1.85 ± 0.16	0.79 ± 0.10	1.29 ± 0.22	86 ± 0.38	85.31 ± 0.75	84.43 ± 0.55
45 + SA	100	7 ± 1.04	5.5 ± 1.05	6* ± 0.00	1.51 ± 0.31	0.73 ± 0.06	0.86* ± 0.27	81.05* ± 0.26	82.41 ± 1.51	81.66 ± 1.32
105 + SA	80	6.6 ± 1.56	4.13* ± 0.23	6* ± 0.00	0.89* ± 0.28	0.43* ± 0.08	0.53* ± 0.12	83.18 ± 0.83	81.92 ± 1.55	80.86 ± 1.13
157.5 + SA	66.67	4.33* ± 0.76	2.27* ± 0.40	4* ± 0.00	0.51* ± 0.03	0.30* ± 0.06	0.24* ± 0.08	82.92* ± 0.19	81.16 ± 1.69	78.54* ± 0.44

Table 1. Germination and seedling growth of peanut seeds sown for 15 days in sawdust saturated with 200 ml Hoagland solution (1/4 strength) supplemented with different concentrations of NaCl without or with 0.1 mM SA at 26 ± 2 °C and 16/8h photoperiod. Values are mean ± SD. * Sign to significant difference between the control and other treatments at $P \leq 0.5$.

After two weeks in hydroponic culture, peanut plants showed reduction of the estimated growth parameters (Table 2). The sensitivity of peanut plant to salt in hydroponic culture was higher than that in the soil, where low NaCl impaired growth parameters when peanut plants were grown in nutrient solution containing relatively low NaCl concentration (45 mM), but vice versa in soil containing the same concentration of NaCl (Hassanein 1999). Under the influence of moderate or high NaCl concentrations, although the root and stem growth parameters were significantly reduced at the seedling stage (Table 1), they showed non-significant reduction when vegetative growth of plants was carried out in hydroponic cultures (Table 2). In general, vegetative growth stage of peanut was more tolerant to salinity than seedling growth stage. In addition, peanut roots in the vegetative growth stage were less adversely affected by high salt concentrations than stems. Many researches illustrated that the inhibition in plant growth under salt stress can be attributed to disturbance in Na^+ and Cl^- ions homeostasis, closure of stomata, and ROS raised production in chloroplasts (Meneguzzo *et al*., 1999; Steduto *et al*., 2000).

In hydroponic cultures, salicylic acid improved the growth of unstressed peanut plants and alleviated the negative effect of NaCl on vegetative growth and water content of different plant organs when plants were subjected to moderate concentration of NaCl (Table 2). Salicylic acid improved the adversely effect of salinity on fresh and dry masses in many plants (Shakirova *et al*., 2003; El-Tayeb, 2005; Baninasab and Baghbanha, 2013).

Polyethylene glycol had a negative effect on seeds germination of peanut especially under relatively high concentrations (Table 3). All seedling growth parameters were significantly reduced by increasing PEG concentration (Table 3). Germination percentage and seedling growth of plants were inhibited by sever stresses due to limitation in water uptake, reduction in enzymes activity, photosynthesis disorder and disturbance in seedling growth (Takel,2000; Kabiri *et al*., 2012). Relatively low PEG concentration (5%) increased root length compared with control. Data in Table 3 indicated that shoot growth was adversely influenced more than root growth under drought stress as was reported previously (Liu *et al*., 2011).

NaCl Coc. (mM)	Root Length (cm)	Stem Length (cm)	No. of leaves/ shoot	Root F.W (g)	Stem F.W. (g)	Leaves F.W. (g)	Root W.C. (%)	Stem W.C. (%)	Leaves W.C. (%)
0.0 (Control)	12.23 ± 1.37	18 ± 1	11 ± 0.00	1.72 ± 0.34	1.19 ± 0.11	2.33 ± 0.32	91.61 ±0 .17	84.78 ± 1.28	86.28 ± 0.66
45	11.07 ± 0.64	14* ± 1.80	10.00* ± 0.00	1.62 ± 0.31	1.08 ± 0.10	2.37 ± 0.05	91.94 ± 0.65	85.13 ± 2.77	84.69 ± 1.56
105	10.57 ± 0.32	11.06* ± 0.50	9.00* ± 0.00	1.37 ± 0.11	0.86 ± 0.13	1.23* ± 0.22	90.94 ± 0.86	79.64 ± 0.66	78.80* ± 1.01
157.5	10.23 ± 0.89	9.93* ± 1.79	8.66* ± 0.57	1.25 ± 0.259	0.84 ± 0.08	1.10* ±0.14	91.20 ± 0.41	83.93 ± 1.85	69.09* ± 1.56
0.0 + SA	12.77 ± 0.80	18.1 ± 0.61	11 ± 0.00	1.64 ± 0.17	1.39 ± 0.11	2.50 ± 0.42	91.94 ± 0.79	88.25 ± 0.28	87.02 ± 1.56
45 + SA	14.03 ± 4.37	13.3* ± 0.3	10* ± 0.00	1.43 ± 0.09	1.10 ± 0.11	2.48 ± 0.46	91.95 ± 0.67	85.19 ± 1.19	84.89 ± 1.07
105 + SA	11.27 ± 0.14	11.87* ± 2.68	8.33* ± 0.57	1.41 ± 0.14	1.004 ± 0.25	1.45* ± 0.25	91.55 ± 0.56	84.52 ± 2.27	83.46 ± 0.69
157.5 + SA	10.73 ± .64	11.47* ± 0.30	7* ± 0.00	1.03* ± 0.06	1.05 ± 0.08	1.02* ± 0.10	89.21*± 0.67	81.09 ± 5.37	75.26* ± 6.03

Table 2. Growth parameters of peanut plants were hydroponically cultured for 15 days in Hoagland solution (1/2 strength) supplemented with different concentration of NaCl in the absence or presence of 0.1 mM SA at 26 ± 2 °C and 16/8h photoperiod. Values are mean ± SD. * Sign to significant difference between the control and other treatments at $P \leq 0.5$

Inclusion of SA with PEG slightly improved seedling growth of peanut grown at 10% PEG. Also, the positive effect of SA on seedling growth appeared in untreated control plants. Under the effect 15% PEG, SA only increased the stem growth and water contents of different plant organs. The stimulating effect of SA on germination and dry weight of water stressed plants may be due to increase antioxidants that protect the plant from oxidative damage (Baalbaki *et al*., 1999; Singh and Usha, 2003). Nemeth *et al*. (2002) found that supplementing root medium with SA inhibited the growth of maize under drought stress.

PEG Conc. (%)	Germination percentage (%)	Root length (cm)	Stem Length (cm)	No. of leaves/shoot	Root F.W (g)	Stem F.W. (g)	Leaves F.W. (g)	Root W.C. (%)	Stem W.C. (%)	Leaves W.C. (%)
0.0 (control)	100	9.1 ± 1.35	8 ± 1.37	7.00 ± 0.00	1.03 ± 0.11	0.75 ± 0.03	1.38± 0.19	84.95± 0.86	83.36 ±0.29	82.77± 0.58
5	100	9.50 ± 1.3	7.27 ± 0.46	6.00* ± 0.00	0.90 ± 0.14	0.61 ± 0.03	0.88* ± 0.21	80.26 ±0.29	81.46± 1.96	81.82 ±1.32
10	100	5.10* ± 0.70	2.40* ± 0.10	5.33* ± 0.57	0.34* ± 0.06	0.19* ± 0.02	0.31* ± 0.09	75.72*±1.33	75.72 ±1.16	73.72*±2.16
15	100	4.67* ± 0.06	0.67* ± 0.15	2.00* ± 0.00	0.25* ± 0.04	0.04* ± 0.01	0.04*±0.008	72.25*±6.89	64.07*± 3.52	72.83*± 0.27
20	66.67	4.5* ± 0.26	0.63* ± 0.05	2.00* ± 0.00	0.05* ± 0.007	0.03*±0.009	0	69.27*±1.003	56.85* ± 0.81	0
25	66.67	0.50* ± 0.10	0	0	0.03* ± 0.01	0	0	67.95*± 2.13	0	0
0.0 + SA	100	9.7 ± 1.04	8.70 ± 1.7	7.00 ± 0.00	1.85* ± 0.16	0.79 ± 0.10	1.29 ± 0.22	86.31± 0.38	85.31± 0.75	84.43±0.55
5 + SA	100	7.33 ± 0.15	4.17* ± 0.05	6.00* ± 0.00	0.49* ± 0.13	0.31* ± 0.09	0.54* ± 0.17	80.58± 0.8	78.96± 1.01	77.86±1.20
10 + SA	100	5.53* ± 0.28	3.13* ± 0.75	5.33* ± 0.57	0.64* ± 0.06	0.36* ± 0.06	0.51* ± 0.08	77.44±2.83	73.95*± 3.14	73.63*±3.09
15 + SA	100	3.53*± 0.41	0.70* ± 0.10	2.00* ± 0.00	0.08* ± 0.02	0.05* ± 0.01	0.035*±0.01	75.37*± 1.45	65.3*± 7.97	74.77±9.56
20 + SA	66.67	1.23* ± 0.25	0.53* ± 0.05	2.00* ± 0.00	0.06*±0.007	0.04* ± 0.01	0	70.09*± 4.37	58.31*± 4.86	0
25 + SA	66.67	0.47* ± 0.21	0	0	0.04* ± 0.01	0	0	45.68*± 3.84	0	0

Table 3. Germination and seedling growth of peanut seeds sown for 15 days in sawdust saturated with 200 ml Hoagland solution (1/4 strength) supplemented with different concentrations of PEG without or with 0.1 mM SA at 26 ± 2 °C and 16/8h photoperiod. Values are mean ± SD. * Sign to significant difference between the control and other treatments at $P \leq 0.5$.

Peanut vegetative growth stage was very sensitive to PEG_{6000}, where, all the cultured plants started to wilt within three days, especially under the influence of relatively high concentration of PEG (25%). Root, stem and leaves fresh masses as well as root and leaves water contents of peanut plants were significantly reduced under the effect of PEG concentrations more than 5% in the absence or presence of SA (Table 4). Peanut was more sensitive to PEG than NaCl during seedling and vegetative growth.These results are in accordance with other reports (Radic and Pevalek-Kozlina 2010; Petrovic *et al*., 2016).It obvious that responses of peanut to saline and drought stresses differed according to developmental stage and the type of stress.

PEG Conc. (%)	F.W. / Root (g)	D.W. / Root (g)	Root W.C. (%)	F.W. / Stem (g)	D.W. / Stem (g)	Stem W.C. (%)	F.W. / Leaves (g)	D.W. / Leaves (g)	Leaves W.C. (%)
0.0 (control)	2.24±0.25	0.17 ± 0.03	93.23 ± 0.20	0.98 ±0.13	0.15 ± 0.02	84.60 ± 1.16	1.15 ±0.17	0.18 ± 0.02	84.63 ±0.59
5	1.22* ± 0.06	0.14 ± 0.008	88.72* ± 0.1	0.68 ± 0.10	0.12 ± 0.03	82.69 ± 2.28	0.86 ± 0.18	0.20 ± 0.02	75.94 ± 2.89
10	1.13* ± 0.06	0.14 ± 0.01	87.49*±1.33	0.63* ± 0.03	0.12 ± 0.01	79.66 ± 3.49	0.76* ± 0.06	0.19 ± 0.02	73.83* ± 0.83
15	1.05* ± 0.10	0.16 ± 0.02	84.85*±0.89	0.55* ± 0.04	0.11 ± 0.01	79.15 ± 1.57	0.48* ± 0.01	0.19 ± 0.003	59.14* ±0.43
20	1.03* ± 0.28	0.16 ± 0.04	83.65*±0.50	0.55* ± 0.08	0.12 ± 0.01	76.91 ± 1.51	0.38* ± 0.09	0.18 ± 0.01	52.05* ± 7.13
25	0.94* ± 0.17	0.18 ± 0.05	81.4* ± 2.49	0.44* ± 0.05	0.12 ± 0.02	71.64* ± 9.46	0.26* ± 0.007	0.16 ± 0.009	38.04* ± 1.86
0.0 + SA	1.45* ± 0.19	0.15 ± 0.01	89.82 ± 0.35	1.09 ± 0.19	0.14 ± 0.03	86.98 ± 1.11	2.11* ± 0.12	0.29* ± 0.02	86.08 ± 0.40
5 + SA	0.98* ± 0.03	0.13 ± 0.004	86.69* ± 0.24	0.87 ± 0.07	0.14 ± 0.02	83.36 ± 1.31	1.35 ± 0.22	0.27 ± 0.01	79.41 ± 3.21
10 + SA	0.99*± 0.28	0.13 ± 0.03	86.48* ± 0.48	0.84 ± 0.13	0.12 ± 0.01	85.69 ± 1.72	0.81 ± 0.11	0.20 ± 0.005	73.93* ± 4.88
15 + SA	0.63* ± 0.09	0.10 ± 0.02	85.03* ± 1.71	0.67* ± 0.03	0.10 ± 0.03	85.03 ± 4.98	0.58* ± 0.13	0.18 ± 0.04	66.91* ± 12.80
20 + SA	0.86* ± 0.11	0.14 ± 0.01	83.54* ± 2.10	0.55* ± 0.15	0.11 ± 0.03	78.41 ± 1.95	0.47* ± 0.10	0.22 ± 0.06	52.68* ± 4.77
25 + SA	0.77* ± 0.12	0.14 ± 0.02	81.28* ± 1.37	0.51* ± 0.05	0.12 ± 0.01	76.11 ± 0.51	0.44* ± 0.10	0.21 ± 0.05	51.70* ± 1.78

Table 4. Growth parameters of peanut plants were hydroponically cultured for 3 days in Hoagland solution (1/2 strength) supplemented with different concentration of PEG in the absence or presence of 0.1 mM SA at 26 ± 2 °C and 16/8h photoperiod. Values are mean ± SD. * Sign to significant difference between the control and other treatments at $P \leq 0.5$.

Esterase isoenzyme forms under the influence of NaCl concentrations is shown in Figure (1A).Staining intensity of esterase bands increased in roots when seedlings were subjected for moderate NaCl concentration (lane R2). Under these conditions new isoenzyme form was detected (lane R2; EST-11) but it was not detected in seedling shoot. Under relatively high NaCl concentration, staining intensity and isoenzyme number decreased roots but increased in shoots of seedlings.The staining intensities of most bands in roots were lower than those of shoots. Two esterase isoenzyme forms (EST-1 and EST-2) were detected in seedling shoots and their staining intensity increased when SA was used (Fig. 1B). In addition, SA induced the expression of new band (EST-10) in seedling roots or shoots. The increase or decrease in isoenzyme density refers to an increase or a decrease in enzyme activity (Khavkin and Zabrodina 1994; Hassanein, 1999).

Esterase isoenzyme expression in hydroponic grown peanut plants under salinity stress was visualized in Figure (2). The staining intensity of esterase isoenzyme forms increased with the increase of salt stress in all plant organs (Fig.2B). In addition, EST-1 and EST-2 were only detected in roots and leaves under the influence of salt stress. It was clear that the response of esterase expression in the different tissues of a plant differed depending on the physiological stage (Hassanein, 1999). Chartzoulakis and Klapaki (2000) stated that, the response of plants to salinity stress differs not only from species to species, but also within a particular species. In comparison to plants grown on NaCl solutions without SA (Fig 2A), application of SA increased the expression of esterase isoenzymes in all plant organs, where the number and staining intensity of isoenzyme forms increased when peanut plants were hydroponically grown in saline solution containing SA (Fig. 2B).

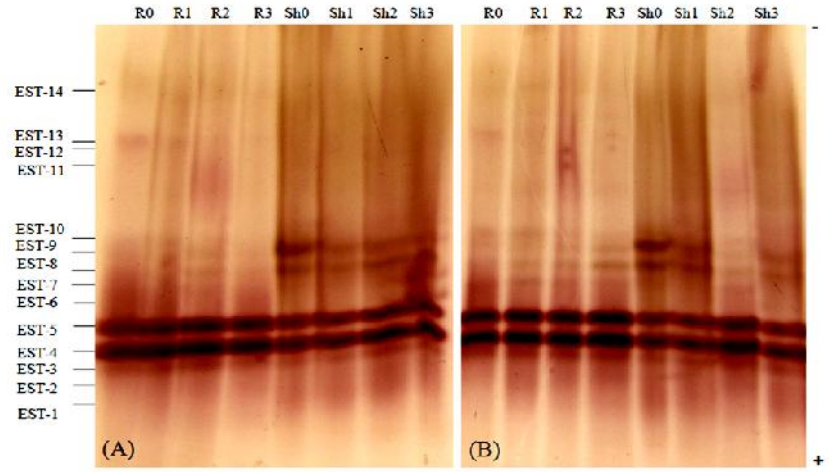

Fig. 1. Native gel electrophoresis for esterase isoenzyme patterns of roots (R) and shoots (Sh) of peanut seedlings grown under the influence of different NaCl concentrations: 0, 45, 105 or 157.5 mM (referred to as 0, 1, 2 or 3, respectively) without (Fig. A) or with (Fig. B) 0.1 mM SA.

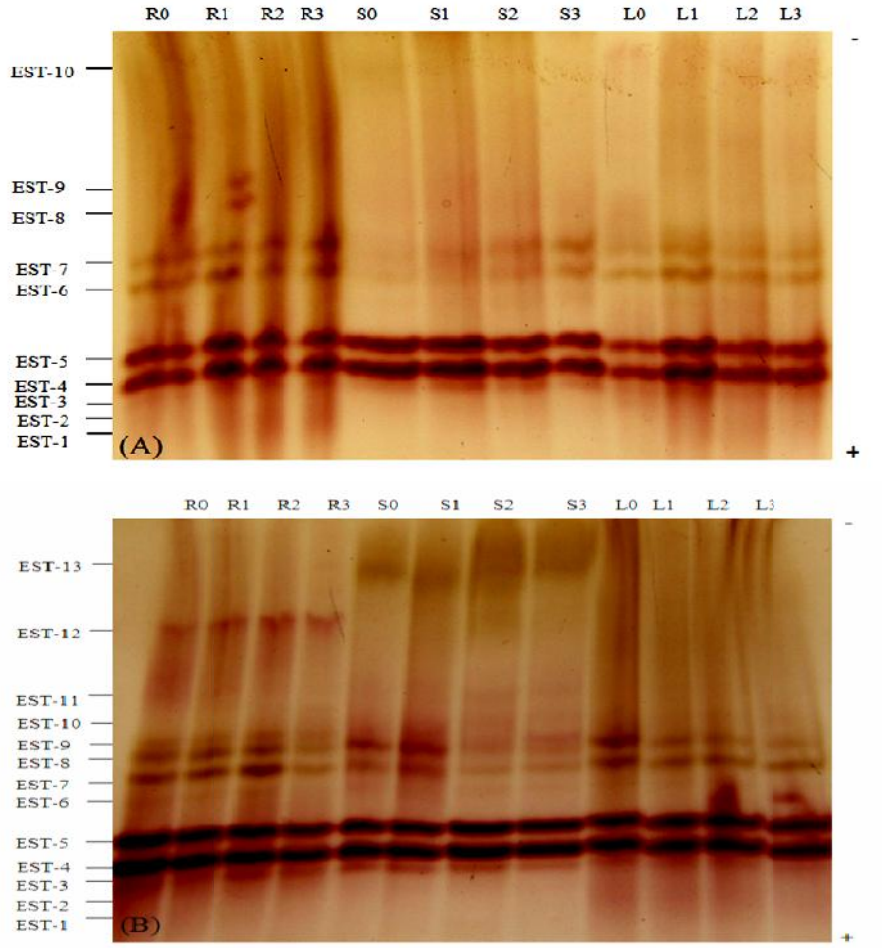

Fig. 2. Esterase isoenzymes profiles of roots (R), stems (S) and leaves (L) of peanut plants grown hydroponically under the influence of different NaCl concentrations: 0, 45, 105 or 157.5 mM (referred to as 0, 1, 2 or 3, respectively) without (Fig. A) or with (Fig. B) 0.1 mM SA.

Drought stress, due to the application of PEG, enhanced esterase expression in roots of peanut seedlings more than the shoots, either in absence or presence of SA (Fig. 3), where some bands were detected in roots (EST-1 and EST-2) but disappeared in shoots. In addition, roots growth parameters were better than those of shoots. Peanut root increased the expression of esterases through: 1) increase the number of esterase isoenzyme forms where EST-1, EST-2 and EST-11 were detected especially under moderate salt stress. 2) the staining intensity of these bands increased under the influence of drought stress. Staining intensities of root bands increased under PEG effect compared to those of control plants, and vice versa in shoots (Fig. 3A). Radic and Pevalek-Kozlina (2010) found that salt-induced some isoesterases as well as mannitol did in roots of *Centaurea ragusina.* When 10 % PEG was used in combination with SA, growth of root, stem and root were improved, the condition which increased the density of some esterase bands (Lanes R2 and Sh2 in Fig 3B). Generally, the staining intensity of esterse bands of shoots increased under the effect of PEG in combination with SA.

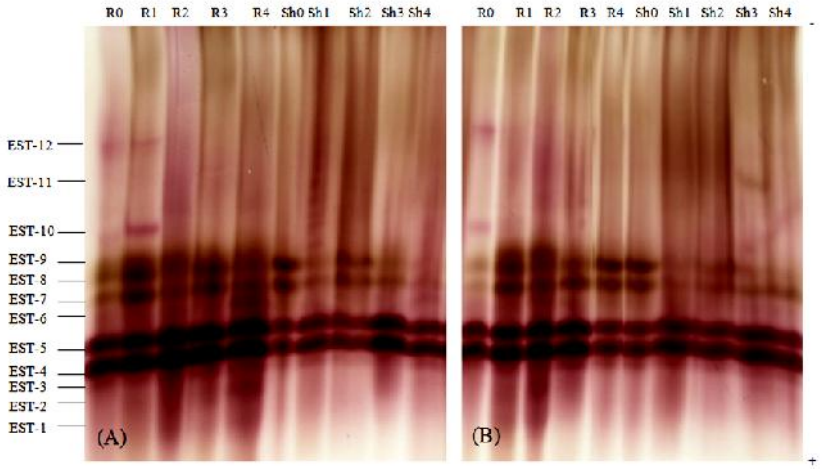

Fig. 3. Esterase isoenzymes patterns of roots (R) and shoots (Sh) of peanut seedlings grown under the influence of PEG different concentrations: 0, 5, 10, 15 or 20% (referred to as: 0, 1, 2, 3 or 4, respectively) without (Fig. A) or with (Fig. B) 0.1 mM SA.

The differential response of different plant organs to PEG stress was observed in esterase patterns of peanut plants that were hydroponically grown in absence or presence of SA (Fig. 4). In SA free hydroponic culture, eight esterase isoenzymes were expressed in roots versus six in stems and five in leaves of untreated control plants. Also, the staining intensity of esterase bands increased under the influence of PEG compared to the control of each organ, similar to salinity. Salicylic acid stimulated the expression of extra esterase isoenzymes in peanut plants cultured hydroponically under the influence of PEG. In general, 10-12 isoesterases in roots and 7-8 isofroms in stems of peanut plants were characterized in presence of SA (Fig. 4B). The appearance of these new isoforms under the presence of SA may be attributed to the potentiality of SA to trigger specific gene expression under drought stress. The increase in esterase activity under stress conditions was considered as a defense mechanism against oxidative damage in plants (Lambert *et al*., 1999; Andres *et al*., 2001).

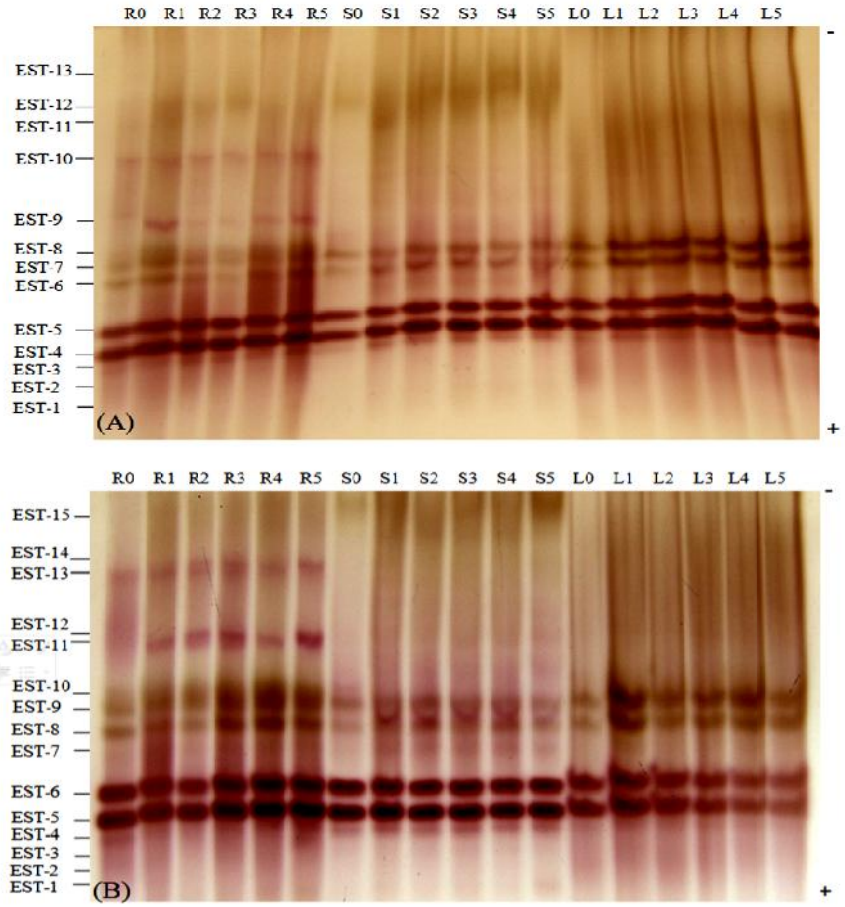

Fig. 4. Native gel electrophoresis foresterase isoenzymes profiles of roots (R), stems (S) and leaves (L) of peanut plantsgrown hydroponically under the influence of 0, 5, 10, 15, 20 or 25% (referred to as 0, 1, 2, 3, 4, or 5, respectively) PEG, without (Fig. A) or with (Fig. B) 0.1 mM SA.

Under studied conditions, the majority of isoesterase bands were purple red and only few ones were dark brown after gel staining with α- and β-naphthyl acetate. This indicated the preference of peanut organs isoesterases toward β-naphthyl acetate, while preference of some others toward α-naphthyl acetate where bands were brown. Radic and Pevalek-Kozlina (2010) observed that the majority of isoesterase bands of *Centaurea ragusina* leaves were dark brown and few were purple red after gel staining with α- and β-naphthyl acetate, while isoesterase bands of roots did not exhibit any preference toward a certain substrate.

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الملخص العربى

إستجابات أيزوإنزيم الأستيريز المختلفة في نبات الفول السوداني للملوحة والجفاف كنتيجة لإستخدام حمض السالسليك

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تم دراسة تأثير الملوحة والجفاف في غياب وكذلك في وجود حمض السالسليك علي نمو نبات الفول السوداني وكذلك تم دراسة التعبير الجيني لإنزيم الإستيريز بالنبات تحت تأثير هذه الظروف. بذور الفول السوداني أستطاعت الإنبات تحت تأثير التركيزات العالية نسبياً من ملح كلوريد الصوديوم (١٥٧.٥ مل مول) أو البولي إيثيلين جليكول (٢٥%). عموما،طور البادرة كان أقل تحملاً لإجهاد الملوحة من طور النمو الخضري للنباتات المنماة في مزارع مائية محتوية علي كلوريد الصوديوم، والعكس حدث عند إستخدام البولي إيثيلين جليكول. علي الرغم من أن إستخدام حمض السالسليك (٠.١ مل مول) ثبط إنبات بذور الفول السوداني، إلا أنه حسن نمو البادرات وكذلك النمو الخضري تحت تأثير التركيز المتوسط من كلوريد الصوديوم (١٠٥ مل مول). حدث نقص معنوي في نمو البادرات وكذلك النمو الخضري لنبات الفول السوداني مع زيادة تركيز البولي إيثيلين جليكول. استخدام حمض السالسليك حسن النمو الخضري للنباتات المعاملة بتركيز ١٠% بولي إيثيلين جليكول. مرحلة النمو الخضري لنبات الفول السوداني كانت أكثر حساسية لإجهاد الجفاف من إجهاد الملح حيث أن النباتات المعاملة بالبولي إيثيلين جليكول ذبلت خلال ثلاثة أيام من الزراعة في المزارع المائية وخاصة في التركيزات العالية نسبياً. التعبير الجيني لإنزيم الإستريز زاد في جذور بادرات نبات الفول السوداني المنماة تحت تأثير التركيز المتوسط من الملح (١٠٥ مل مول) وتركيز ١٠% من البولي إيثيلين جليكول وتم الإستدلال علي هذه الزيادة من خلال زيادة عدد الباندات وزيادة كثافة بعض الباندات. أيضاً النباتات المنماة في المزارع المائية في مرحلة النمو الخضري تحت تأثير التركيزات العالية نسبياً من الملح والبولي إيثيلين جليكول أظهرت زيادة في نشاط إنزيم الإستيريز واستدل علي هذه الزيادة أيضاً من خلال زيادة عدد أشكال الأيزوإنزيمات للإستيريز وكذلك كثافة الباندات. إستخدام حمض الساسليك زود عدد باندات وكذلك كثافة باندات إنزيم الإستيريز وذلك في طور نمو البادرات وطور النمو الخضري لنباتات الفول السوداني المنماة تحت تأثير التركيزات العالية من الملح والبولي إيثيلين جليكول.