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Alleviation the Salinity Stress on Metabolic Contents of *Hordeum vulgare* L. and *Phaseolus vulgaris* L. Using some Plant Phenols

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THIS research provides an updated and comprehensive overview on alleviation of salt stress on *Hordeum vulgare* L. (barley) and *Phaseolus vulgaris* L. (bean) by plant natural phenols (POH). The effect of exogenously application of POH on metabolic contents of barley and bean under saline stress was investigated. Increased salinity stress significantly reduced total soluble sugars and total protein contents of barley and bean seedlings. Pre-treatment of barley grains or bean seeds with POH stimulated the accumulation of total soluble sugars and total protein of seedlings as compared with the corresponding salinity level. Increased salinity stress and pre-treatment with POH increased the total phenols in barley and bean seedlings. Meanwhile salinity stress induced accumulation of proline in barley and bean seedlings and pre-treatment with POH decrease proline content as compared with those of the corresponding salinity level. This is indication that salt stress on plants was improved by POH pre-treatment through decreasing proline synthesis or accumulation. Also, the seedlings pre-treated with POH showed new protein bands. Further analyses of this mutant and its genetic construction are needed to for more understanding of its tolerant ability to salt stress. The data provided evidence that pre-treatment with phenols reduced the adverse effects of salt stress on barley and bean plants, and might play a key role in providing stress tolerance by modulating oxidative stress of salinity.

Keywords: Barley, Bean, Salt stress, Phenolic compounds, Sugars, Proteins, Total phenols and proline.

Salinity is one of the major abiotic stresses that adversely affect plant productivity and quality (Mehr *et al.*, 2012). It was estimated that up to 20% of irrigated lands in the world are affected by different levels of salinity (Mostafazadeh-Fard *et al.*, 2007). Many crop species are sensitive to salinity and negative impacts on agricultural production (Zorb *et al.*, 2004). Salinity stress limits plant growth by adversely affecting various physiological and biochemical processes like photosynthesis, antioxidant phenomena, nitrogen metabolism, ion homeostasis

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(Misra *et al.*, 2006 and Ashraf, 2004), proline metabolism and osmolytes accumulation (Misra and Gupta, 2005). Several investigators have demonstrated that protein and amino acids metabolism is strongly influenced by changes in the salinity concentrations (Li *et al*., 2010). In particular, different amino acids are accumulated at different rates under a salt-stressed condition; for example, proline which forms a minor component of the pool of free amino acids in glycophytes, accumulates under stress conditions (Khedr *et al*., 2003).

Survival under these stressful conditions depends on the plant's ability to perceive the stimulus, generates and transmits signals and instigates biochemical changes that adjust the metabolism accordingly (Duan *et al.*, 2008 and Dolatabadian & Saleh, 2009). Proteins and proline accumulation is one of the most frequently reported modifications induced by salinity in plants (Girija *et al.*, 2002 and Misra & Gupta, 2005) and involved in stress resistance mechanisms. Petridis *et al.* (2012) showed that the total phenol and the secoiridoid concentrations increased in the salt-treated plants when they exposed to full sunlight, but not in the shaded side of the olive trees.

One approach for gaining a better understanding of the mechanisms by which plants can respond to salt stress is to study those proteins that are specifically accumulated after exposure of the plants to salinity (Parida *et al*., 2004). The resolving power of one-dimensional polyacrylamide gel electrophoresis allows the detection of minor differences between protein patterns. This has been exploited in a number of studies that have been conducted (Radwan *et al.*, 2013).

Antioxidants are compounds that can delay or inhibit the oxidation by inhibiting the initiation or propagation of oxidizing chain reactions (Ashraf and Harris, 2004). Recently, natural phenolic compounds have attracted special interests (Dai and Russell, 2010) and play a vital role in the prevention of oxidation process (Maróstica *et al.*, 2010). The biological mechanisms of these attributed to their antioxidant properties through their ability to scavenge free radicals, break radical chain reactions, directly reducing peroxides and stimulating the antioxidative defense enzyme activities (Maróstica *et al.*, 2010).

The antioxidant activity of phenolic compounds is mainly due to their redox properties in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994). It has been proposed also that phenolic compounds can be mediated by the following mechanisms: (1) scavenging radical species such as ROS/RNS, (2) suppressing ROS/RNS formation by inhibiting some enzymes or chelating trace metals involved in free radical production and (3) up regulating or protecting antioxidant defense (Cotelle, 2001).

Spinach (*Spinacia oleracea* L.), amaranth *(Amaranthus viridis* L.), tomato *(Lycopersicon esculentum* Mill.) and cabbage (*Brassica oleracea* L. v. *capatate*) are among the important plants for natural phenols (Deef *et al*., 2013).

The aim of the present work was to improve the salt tolerance of barley and bean plants by exogenous pre-treatments of grains or seeds with natural plant phenols. To achieve these goals, sugars, proteins, phenols, proline and protein profile were analyzed in barley and bean seedlings growing at four phases of NaCl (0, 50, 100 and 200 mM).

Material and Methods

The tested species

Hordeum vulgare L. (barley) is a widely adaptable cereal crop. Barley is more tolerant of soil salinity than wheat, which might explain the increase of barley cultivation especially in arid regions (Komatsuda *et al.*, 2006).

Phaseolus vulgaris L. (bean) is a member of the pulses family (Papilionaceae), extremely sensitive to salinity, and suffers yield losses at soil salinity less than 2 dS m^{-1} (FAOSTAT, 2009). It produces an osmolyte called proline, which stabilizes cell membranes, making them less permeable for adverse ions (Sando and Barrington, 2008).

Source of plant phenols

Spinach (*Spinacia oleracea* L.), belongs to family Amaranthaceae- subfamily: Chenopodioideae. Carotenoids, ascorbic acid, flavonoids, p-coumaric acid derivatives and phenolic acids are the antioxidant components of the aqueous extract of spinach leaves (Andjelkovic *et al.*, 2008).

Amaranth (*Amaranthus viridis* L.) (Amaranthaceae) is a cosmopolitan species, growing under a wide range of climatic conditions and is able to produce grains and leafy edible vegetables.

Tomato (*Lycopersicon esculentum* Mill.) or the edible, typically red, fruit which it bears. Among the vegetables, tomatoes represent the predominant source of antioxidants and besides the Carotenoides (lycopene, β-carotene and lutein), the flavonoids have been confirmed as a group of polyphenols important in conferring antioxidant benefits (Slimestad and Verheul, 2005). The flavanone, naringenin (4′, 5, 7-trihydroxyflavanone) and the hydroxycinnamate, chlorogenic acid (5-caffeoylquinic acid) are among the most abundant phenolics in tomato (Luthria *et al.*, 2006).

Cabbage is a popular cultivar of the species *Brassica oleracea* L. v. *capitata* Family Brassicaceae (Cruciferae) and is a leafy green vegetable. Flavonoids and other phenolics are contributed to cancer preventive properties of cabbage (Marchand, 2002 and Galati & O'Brien, 2004).

Collection of plant materials (source of phenols)

Four plant species namely; spinach (*Spinacia oleracea* L.), amaranth *(Amaranthus viridis* L.), tomato *(Lycopersicon esculentum* Mill.) and cabbage (*Brassica oleracea* L. *v. capatate*); (Egyptian origin cultivars) at Sharkia Governorate were used in this study. Fresh plant shoots were collected in July-September, 2011. The identification of plants was confirmed by a specialist.

Preparation of phenolic extracts

The shoot system of plants collected were harvested, dried and ground to fine powder by a Kenwood multi-mill. Phenolic compounds were extracted according to the method described by Sousek *et al.* (1999). The plant materials were chopped into 1 cm long pieces and dried at 50°C for 24 hr. Dry aerial plant material was extracted in Soxhlet apparatus with methanol for 30 min the rate of 1g dry weight per 100 ml. The extracts were evaporated at 40 ^{0}C under vacuum to dryness and the residue was dissolved in H_2O at rate of 1g dry weight per 100 ml, filtered and put at a refrigerator until photometric determination of total phenols and used in treatments of barley grains and bean seeds.

Seeds culture and treatments

Barley grains and bean seeds were surface-sterilized for 5 min in sodium hypochlorite solution (0.5%) and then were rinsed with distilled water. Sterilized grains or seeds were divided into five groups, one soaked in water and the other soaked in four different phenolic extracts (Sp. Ext. = spinach phenolic extract, Am. Ext. = Amaranthus phenolic extract, To. Ext. = tomato phenolic extract, Ca. Ext. = cabbage phenolic extract) for 24 hr. Then ten (grains or seeds) were sowed in a Petri dish under conditions of 26/18°C day/night temperature and natural light. Treatments supplied in four NaCl levels (0, 50, 100 and 200 mM) for each group.

Analysis

After 10 days of salinity treatments the following analysis were carried out to the germinated seedlings:

1. Total soluble sugars (TSS)

Total soluble sugars (TSS) of barley and bean seedlings were extracted by overnight submersion of dry tissue in 10 ml of 80% (v/v) ethanol at 25°C with periodic shaking, and centrifuged at 6000 rpm. The supernatant was evaporated till completely dried then dissolved in a known volume of distilled water (Homme *et al*., 1992). TSS were analyzed by reacting of 0.1 ml of ethanolic extract with 3.0 ml freshly prepared anthrone (150 mg anthrone + 100 ml 72% H_2SO_4) in boiling water bath for ten minutes and reading the cooled samples at 625 nm using Spekol Spectrocololourimeter VEB Carl Zeiss.

Total proteins

Total protein was determined according to the method described by Bradford (1976) with bovine serum albumin as a standard. An amount of 2 gm of seedling samples were grinded in mortar with 5ml of phosphate buffer (pH 7.6) and was then centrifuged at 8000 rpm for 20 min. 30µl of different samples were mixed with 70µl of distilled water then add 2.9 ml of Coosmassic Brillaint Blue solution and mixed thoroughly. The tubes were incubated for 5 min at room temperature and absorbance at 600 nm was recorded against the reagent blank. A standard curve of Absorbance (600 nm) versus Concentration (ug) of protein was calculated.

2. *Total phenolic contents*

POH contents in the barley and bean seedlings were determined with the Folin-Ciocalteu reagent according to the method of Singleton *et al.* (1999) using gallic acid as a standard. Methanolic extract (0.5 ml), 0.5 ml of Folin-Ciocalteu reagent, 10 ml of 7.5% sodium carbonate and deionized water were added to a final volume of 25 ml. After 1 hr, the absorbance of the sample was measured at 725 nm against a blank by spectrophotometer. Gallic acid was used as the standard for preparing the calibration curve. Results were expressed as mg of gallic acid equivalent per gram of fresh weight.

3. Proline

1 gm of samples was extracted with phosphate buffer (pH 7.6) and was then centrifuged at 8000 rpm for 20 min. Proline was assayed according to the method described by Bates *et al*. (1973). 2ml of extract, 2ml of acid ninhydrin and 2ml of glacial acetic acid were added and incubated for 1 hr in a boiling water bath followed by an ice bath. The absorbance was measured at 520 nm using Spekol Spectrocololourimeter VEB Carl Zeiss. A standard curve was obtained using a known concentration of authentic proline.

4. Protein extraction for SDS-PAGE

For SDS-PAGE, seedlings tissues of each sample were ground to powder under liquid nitrogen and melted in ice-cold extraction buffer (50 mM Tris-HCl, pH 8, 10 mM NaCl, 1% SDS, 5% 2-mercaptoethanol, 0.1 mM PMSF, 0.1 mM DTT), followed by centrifugation at 10000 rpm at 4 ^{o}C for 15 min. Protein content of the clear supernatants obtained after centrifugation were stored at -20 ^{0}C until used.

5. One-dimensional SDS-PAGE

Proteins, 30 µg of each sample, were separated by SDS-PAGE according to the method of Laemmli (1970). The separation was performed with a 10% separating gel and a 4% stacking gel using protein vertical electrophoresis unit (Hoefer Scientific Instruments). Electrophoresis was started at 10 mA constant current until the tracking dye entered the separating gel and continued at 25 mA until the tracking dye reached the end of the gel. Protein subunit bands were stained with coomassie blue R-250 by standard techniques. The protein marker from Sigma was used. The molecular weight of standard protein (in kDa) as follows: 7, 15, 20, 25, 35, 50, 70, 100, 140 and 240 kDa (10 bands).

Statistical analysis

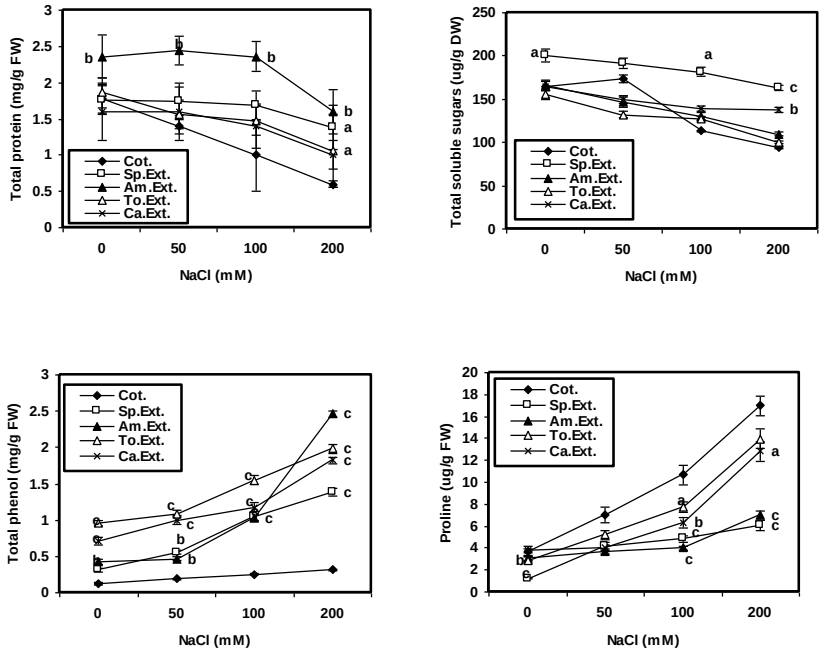
All data are reported as mean ± standard deviation (±SE) for the three independent samples (n=3). Analysis of variance and significant differences among means were tested by one-way ANOVA using the COSTAT computer package (Snedecor and Cochran, 1980).

Results and Discussion

Effect of some plant phenols on metabolic contents of barley and bean under salt stress

1. Total soluble sugars

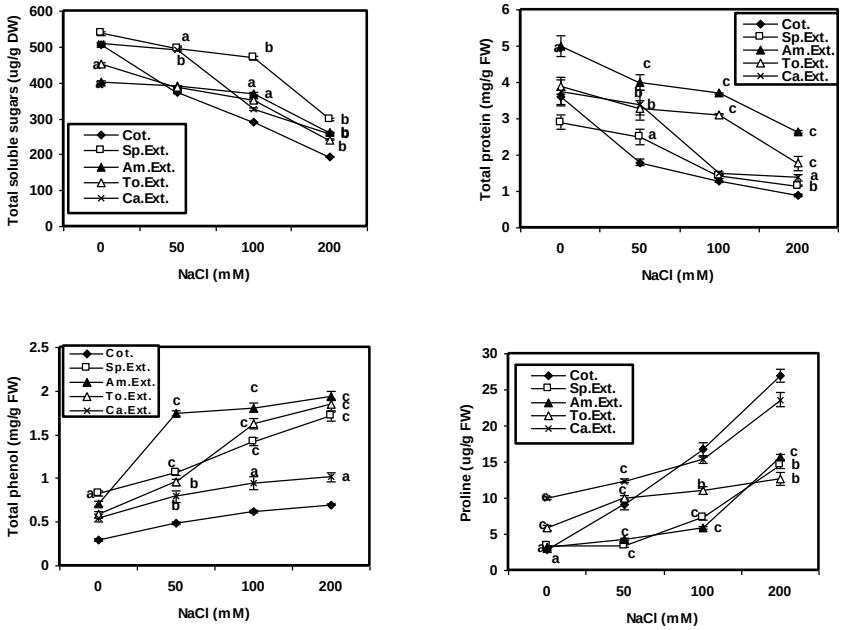
Increased salinity levels from 0 - 200 mM NaCl significantly reduced total soluble sugars contents of barley and bean seedlings (Fig. 1&2). The percentages of decrease were 43% in barley and 62% in bean seedlings respectively. Reduction in sugars accumulation was recorded by other authors (Kafiet *et al*., 2008, Younis *et al*., 2008 and Hassanein *et al*., 2009a, b). NaCl may inhibit photosynthetic activity or increase partial utilization of sugars (Hassanein *et al*., 2009a).



Cot. = Control, Sp. Ext. = Spinach phenolic extract, Am. Ext. = Amaranth phenolic extract, To. Ext. = Tomato phenolic extract, Ca. Ext. = Cabbage phenolic extract.

Means within a column followed by significantly different according to Fisher's, a=Significant at P< 0.5 . b=Significant at P< 0.1 . c=Significant at P< 0.05.

Fig. 1. Effect of some plant phenols on metabolic contents of barley (*Hordeum vulgare* L.) under salt stress. Values are the means ± SEM of three replicated measurements.



Cot. = Control, Sp. Ext. = Spinach phenolic extract, Am. Ext. = Amaranthus phenolic extract, To. *Ext. = Tomato phenolic extract, Ca. Ext. = Cabbage phenolic extract.*
Means within a column followed by significantly different according to Fisher's, a=Significant at P< 0.5 . b=Significant at P< 0.1 . c=Significant at P< 0.05.

Fig. 2. Effect of some plant phenols on metabolic contents of bean (*Phaseolus vulgaris* L.) under salt stress. Values are the means ± SEM of three replicated measurements.

Pre-treatment of grains or seeds with POH increased the total soluble sugars of seedlings compared with the non-treated at the same salinity level. Total soluble sugars accumulated more in barley and bean seedlings at spinach phenolic pre-treatment compared with other POH treatments (Fig. 1&2). Pre-treatment with POH induce salt tolerance of other important crop cultivars (Waseem *et al*., 2006), throw either increasing endogenous levels of certain phytohormones and activation of sugar synthesis (Hassanein *et al*., 2009b) or osmotic adjustment by conferring some desiccation resistance to plant cells (Rady *et al.,* 2011).

According to Jouve *et al.* (2004), carbohydrates may act as ROS scavengers and contribute to increase in membrane stabilization. Soluble sugars and proline protect the plant cell by balancing the osmotic strength of cytosol with that of vacuole and external environment (Radi *et al*., 2013) and interact with cellular macromolecules such as enzymes and stabilize the structure.

2. Total proteins

Gradual decrease in the total proteins content in both barley and bean seedlings with salinity stress (Fig.1& 2). These results can be attributed to the decrease in protein synthesis and/or to the increase in its degradation (Bassouny *et al.*, 2008). Saline stress cause nutrient imbalance, *i.e.*, reduce uptake of potassium ions by plant roots. Potassium loss causes diminished plant growth and development due it's necessarily for protein synthesis (Chen *et al*., 2007). If the stress is prolonged, it could affect protein synthesis and eventually cause it to decline (Ayala-Astorga and Alcaraz-Meléndez, 2010).

Pre-treatment of grains or seeds with POH induced significant increases in total protein contents of seedlings under all salinity levels. The highest total protein in barley and bean seedlings at amaranth phenols treatment (Fig.1&2). Phenolic compounds act as antioxidants that protect plants against the damaging effects of increased ROS levels due to salt stress (Petridis *et al.,* 2012). Phenolic compounds have the ability of phenoxide ion delocalize. The phenoxide ion can lose a further electron to form the corresponding radical which can also delocalize. In reference to this property, phenolic compounds have radical scavenging and antioxidant activity (Maestri *et al.*, 2006).

3. Total phenols

Increased salinity levels from 0 - 200 mM NaCl produce an increase in the percentages of total phenols by 200% in barley and 220% in bean seedlings respectively. The data obtained revealed that the pre-treatment with POH stimulated the accumulation of total phenols as compared with the corresponding salinity level. The highest total phenols accumulated in barley and bean seedlings at amaranth phenolic pre-treatment (Fig. 1 & 2). Petridis *et al.* (2012) showed that the salinity stress increased the total phenol of the trees. Phenols play a significant role in the regulation of plant metabolic processes and overall plant growth (Lewis and Yamamoto, 1990). The obtained data are in good agreement with those obtained by Mohamed and Aly (2008) on onion plant and El- Hariri *et al*. (2010) on flax plant. Phenols act as a substrate for many antioxidants enzymes, so, it mitigates the salinity stress injuries (Lewis and Yamamoto, 1990). In this connection phenol protect cells from potential oxidative damage and increase stability of cell membrane (Burguieres *et al*., 2006). Moreover, Sánchez-Rodríguez *et al*. (2011) recorded that an accumulation of phenolic compounds in response to abiotic stress. This would beneficial to achieve acclimatization and tolerance to salt stress, since many plant phenols have been considered to be the main lines of cell acclimatization against stress in plant (Hassanein *et al*., 2009a).

4. Proline

Data recorded in the present study (Fig. 1 &2) indicate that salt stress induced accumulation of proline in barley and bean seedlings. Pre-treatment with POH decrease proline content as compared with those of the corresponding salinity

level. Strong correlation between proline content and the capacity to survive under the environmental salinity (Misra and Gupta, 2005 and Munns & Tester, 2008)). Similar results have been reached by Khattab (2007) and Sadak *et al.* (2010) on different plant species. The higher level of proline content may be due to expression of gene encoding key enzymes of proline synthesis and low activity of the oxidizing enzymes which is controlled by osmotic and salinity stress (Amini and Ehasapour, 2005). Proline also can play a role as protective agent for cytoplasmic enzymes (Rady *et al.,* 2011) and/or scavenging hydroxyl radicals (Hoque *et al*., 2007). In seedlings of both plants pre-treated with phenol extracts and subjected to saline stress, the proline increment was much smaller (about 60%). Hare *et al.* (1998) have contended that proline content increases when an injury to plant tissue. Possibly in seedlings treated with POH and subjected to saline stress, where growth is greater and plant status better, damage is less and therefore proline levels are hardly increased with respected to the control seedlings. Thus, it could be suggested that POH increase the test plant salt tolerance by decrease in proline content. This means that the inhibitory effect of salt stress on the tested plants was improved by POH treatments through decreasing proline synthesis.

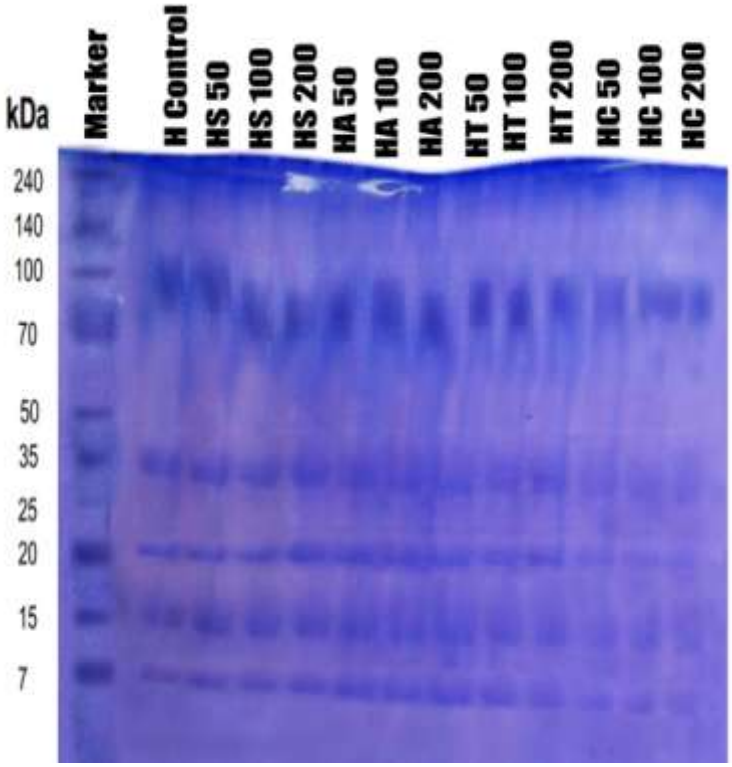
Effect of some plant phenols on protein electrophoretic pattern of barley and bean under salt stress

Electrophoretic patterns of barley and bean seedlings proteins were analyzed by SDS-PAGE as shown in Fig 3 &4. The results showed that the protein profiles of barley pre-treated with POH grow under salt stress represented by 5 major and common distinct bands with molecular weight of 7, 15, 20, 35 and 100 kDa (Fig. 3). The electrophoretic analysis of barley seedlings protein under salt stress shows minor changes between spinach pre-treated POH and other POH pre-treatments. 70 kDa band appear for barley at spinach pre-treated POH only (Fig. 3).

The protein profiles of bean pre-treated with POH grow under salt stress represented by 6 major and common distinct bands with molecular weight of 15, 20, 35, 50, 70 and 140 kDa (Fig. 4). New band of 7 kDa appear at spinach and amaranth POH pre-treated bean seedlings grow under salt stress.

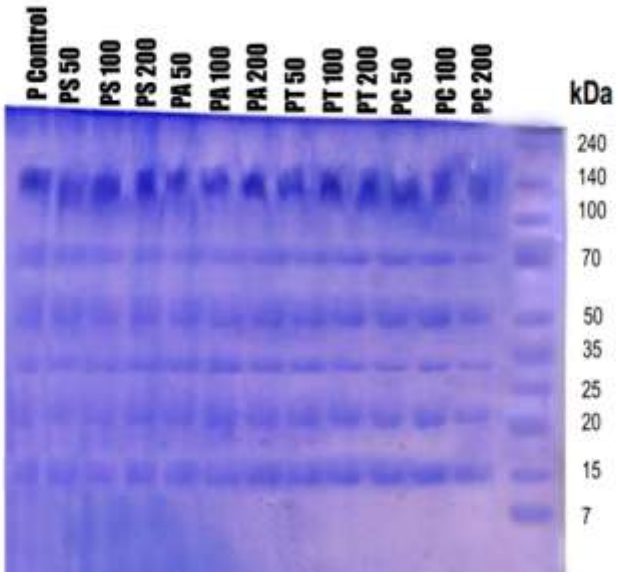
Protein electrophoresis is considered a reliable, practical and reproducible method because proteins are the third hand copy of genomic DNA and largely independent of environmental fluctuations (Javaid *et al*., 2004 and Iqbal *et al*., 2005). The obtained results (Fig .3&4) shows that bands of 15, 20 and 35 kDa are common between barley and bean seedlings under salinity stress. Garcia *et al*. (2005) obtained protein bands with molecular weight 20 and 35 kDa on SDS-PAGE related to stress. Also Chourey *et al*. (2003) demonstrated that salinity stress was able to trigger the accumulation of several major stress proteins. They also stated that the accumulation levels of these proteins correlated with stress tolerance in the various plant species, suggesting protective roles under osmotic stress and that recovery from salt stress was consistently accompanied by

degradation of the salt-stress induced proteins. Parida *et al*. (2004) reported that SDS-PAGE analysis showed nearly identical protein profiles in control and salt treated samples which suggest that salt did not alter protein synthesis or proteolytic activity. The decrease in soluble protein of high salinity may be due to a protein break-down or to an alteration in the incorporation of amino acids into proteins. The appear of new protein bands for barley and bean at spinach and amaranth pre-treated POH may be signal for plants tolerance to salt stress (Radwan *et al.*, 2013).



H Control = barley Control, HS 50= barley treated by spinach POH at 50 mM NaCl, HS 100= barley treated by spinach POH at 100 mM NaCl, HS 200= barley treated by spinach POH at 200 mM NaCl; HA 50= barley treated by amaranth POH at 50 mM NaCl, HA 100= barley treated by amaranth POH at 100 mM NaCl, HA 200= barley treated by amaranth POH at 200 mM NaCl; HT 50= barley treated by tomato POH at 50 mM NaCl, HT 100= barley treated by tomato POH at 100 mM NaCl, HT 200= barley treated by tomato POH at 200 mM NaCl; HC 50= barley treated by cabbage POH at 50 mM NaCl, HC 100= barley treated by cabbage POH at 100 mM NaCl, HC 200= barley treated by cabbage POH at 200 mM NaCl.

Fig. 3. Effect of some plant phenols on protein electrophoretic pattern of barley (*Hordeum vulgare* L.) under salt stress.



P Control = bean Control, PS 50= bean treated by spinach POH at 50 mM NaCl, PS 100= bean treated by spinach POH at 100 mM NaCl, PS 200= bean treated by spinach POH at 200 mM NaCl; PA 50= bean treated by amaranth POH at 50 mM NaCl, PA 100= bean treated by amaranth POH at 100 mM NaCl, PA 200= bean treated by amaranth POH at 200 mM NaCl; PT 50= bean treated by tomato POH at 50 mM NaCl, PT 100= bean treated by tomato POH at 100 mM NaCl, PT 200= bean treated by tomato POH at 200 mM NaCl; PC 50= bean treated by cabbage POH at 50 mM NaCl, PC 100= bean treated by cabbage POH at 100 mM NaCl, PC 200= bean treated by cabbage POH at 200 mM NaCl.

Fig. 4. Effect of some plant phenols on protein electrophoretic pattern of bean (*Phaseolus vulgaris* L.) under salt stress.

References

Amini, F. and Ehsanpour, A.A. (2005) Soluble protein, Carbohydrates and Na^+ K^+ changes in two tomatos (*Lycopersicum esculentum* Mill.) cultivars under in vitro salt stress. *Ameri. J. of Bioch. and Biotech.* **1** (4): 212-216.

Andjelkovic, M., Bunea, A., Socaciu, C., Bobis, O., Neacsu, M. and Verhe, R. (2008) Total and individual carotenoids and phenolic acids content in fresh, refrigerated and processed spinach (*Spinacia oleracea* L.). *Food Chem.* **108**: 649–56.

Ashraf, M. (2004: Some important physiological selection criteria for salt-tolerance in plants. *Flora* **199**: 361-376.

Ashraf, M. and Harris, P.J.C. (2004) Potential biochemical indicators of salinity tolerance in plant. *Plant Sci.* **166**: 3-16.

Ayala-Astorga, G., I. and Alcaraz-Meléndez, **L. (2010):** Salinity effects on protein content, lipid peroxidation, pigments, and proline in *Paulownia imperiali*s (Siebold & Zuccarini) and *Paulownia fortunei* (Seemann & Hemsley) grown *in vitro*. *Environmental Biotechnology* **13** (5): 15, 20-28.

Bassouny, F. M., Hassanin, R.A., Baraka, D.M. and Khalil, R.R. (2008) Physiological effects of nicotinamide and ascorbic acid on *Zea mays* plant grown under salinity stress II- Changes in nitrogen constituents and certain inorganic cations. *Australian J. Basic and Applied Sci*. **2**(3): 350-359.

Bates, L.S., Waldern, S.P. and Teare, I.D. (1973) Rapid determination of free proline for water-stress studies. *Plant Soil* **39**: 205–207.

Bradford, M.M. (1976) A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. *Anal. Biochem.* **72**: 248-54.

Burguieres, E., McCxue, P., Kwon, Y. and Shelty, K. (2006) Effect of vitamin C and folic acid on seed vigour response and phenolic-antioxidant activity. *Biores. Technol.* **95**: 1393-1404.

Chen, Z., Cuin, T. A., Zhou, M., Twomey, A., Naidu, B., P. and Shabal, A. (2007) Sergey. Compatible solute accumulation and stress-mitigating effects in barley genotypes contrasting in their salt tolerance. *Journal of Experimental Botany* **58** (15-16): 4245-4255.

Chourey, S., Apte, S.K., Chourey, K. and Ramani, S. (2003) Accumulation of LEA proteins in salt (NaCl) stressed young seedlings of rice (*Oryza sativa* L.) cultivar Bura Rata and their degradation during recovery from salinity stress. *J. Plant Physiol.* **160**: 1165-1174.

Cotelle, N. (2001) Role of flavonoids in oxidative stress. *Curr. Top. Med. Chem.* **1**: 569-590.

Dai, J. and Russell, J. M. (2010) Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules* **15**: 7313-7352.

Deef, H. E., Taleb, S. S. and Gareeb, R. M. (2013) Alleviation the salinity stress on the growth and antioxidative enzymes of *Hordeum vulgare* and *Phaseolus vulgaris* using some plant phenols. *Environmental Conf.*, Faculty of Science, Zagazig Univ. : 156-179.

Dolatabadian, A. and Saleh, R.J. (2009) Impact of exogenous ascorbic acid on antioxidant activity and some physiological traits of common bean subjected to salinity stress. *Not. Bot. Hort. Agrobot. Cluj*. **37**(2): 165-172.

Duan, J., Li, J., Guo, S. and Kang, Y. (2008) Exogenous spermidine affects polyamine metabolism in salinity-stressed *Cucumis sativus* roots and enhances short-term salinity tolerance. *J. Plant Physiol.* **165**: 1620-1635.

El- Hariri, D.M., Sadak, M.S.H. and El-Bassiouny, H.M.S. (2010) Response of flax cultivars to ascorbic acid and α – tocopherol under salinity stress conditions. *Inter. J. Acad. Res.* **2** (6): 101-109.

FAOSTAT (2009) Food and Agricultural Organization of the United Nations, accessed November 5.

Galati, G. and O'Brien, P. J.(2004) Potential toxicity of flavonoids and other dietary phenolics: Significance for their chemopreventive and anticancer properties. *Free Radical Biol. Med.* **37**: 287-303.

Garcia, N.A.T., Iribarne, C., Lopez, M., Herrera-Cervera, J.A. and Lluch, C. (2005) Physiological implications of trehalase from *Phaseolus vulgaris* root nodules: Partial purification and characterization. *Plant Physiol. Biochem.* **43**: 355-361.

Girija, C., Smith, B.N. and Swamy, P.M. (2002) Interactive effects of sodium chloride and calcium chloride on the accumulation of proline and glycinebetaine in peanut (*Arachis hypogaea* L.). *Environ. Exper.Bot.* **47**: 1–10.

Hare, P. D., Cress, W. A. and Staden, J. V. (1998) Dissecting the roles of osmolyte accumulation during stress. *Plant Cell Environ.* **21**: 535-553.

Hassanein, R.A., Bassuony, F.M., Baraka, D.M. and Khalil, R.R. (2009a) Physiological effects of nicotinamide and ascorbic acid on *Zea mays* plant grown under salinity stress. I-Changes in growth, some relevant metabolic activities and oxidative defense systems. *Res. J. Agricult Biol. Sci.* **5**: 72-81.

Hassanein, R.A., Hassanein, A.A., Haider, A.S. and Hashem, H.A. (2009b) Improving salt tolerance of *Zea mays* L. plant by presoaking their grains in glycine betaine. *Aust. J. Basic Appl. Sci.* **3**: 928-942.

Homme, P.M.; Gonzalez, B. and Billard, J. (1992) Carbohydrate content, frutane and sucrose enzyme activities in roots, stubble and leaves of rye grass (*Lolium perenne* L.) as affected by sources/link modification after cutting. *J. Plant Physiol.* **140**: 282-291.

Hoque, M.D.A., Okuma, E., Banu, M.N.A., Nakamura, Y., Shimoishi, Y. and Murata, Y. (2007) Exogenous proline mitigates the determintal effects of salt stress more than exogenous betaine by increasing antioxidant enzyme activities. *J. Plant Physiol.* **164**: 553-561.

Iqbal, S.H., Ghafoor, A. and Ayub, N. (2005) Relationship between SDS-PAGE markers and *Ascochyta* blight in chickpea. *Pakistanian Journal of Botany* **37**: 87-96.

Javaid, A., Ghafoor, A. and Anwar, R. (2004) Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. *Pakistanian Journal of Botan.* **36**: 25-29.

Jouve, L., Hoffmann, L. and Hausman, J. F. (2004) Polyamine, carbohydrate, and proline content changes during salt stress exposure of Aspen (*Populus tremula* L.): involvement of oxidation and osmoregulation metabolism. *Plant Biol.* **6**: 74-80.

Kafiet, M.; Stewart, W.S. and Borland, A.M. (2008) Carbohydrate and proline contents in leaves, roots and apices of salt tolerant and salt-sensitive wheat cultivars. *Russian J. Plant Physiol.* **50**: 155-162.

Khattab, H. (2007) Role of glutathione and polyadenylic acid on the oxidative defense systems of two different cultivars of canola seedlings grown under saline conditions. *Austr. J. of Basic and Applied Sci.* **1**(3): 323-334.

Khedr, A.H.A., Abbas, M.A., Abdel Wahid, A.A., Quick, W.P. and Abogadallah, G.M. (2003): Proline induces the expression of salt-stress-responsive proteins and may improve the adaptation of *Pancratium maritimum* L. to salt-stress. *J. Exp. Bot.* **54**: 2553-2562.

Komatsuda, T., Pourkheirandish, M, He, C., Azhaguvel, P., Kanamori, H., Perovic, D., Stein, N. and Graner, A. (2006) Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene. *Proceedings of the National Academy of Sciences of the United States of America* **104** (4): 1424–1429.

Laemmli, U.K. (1970): Cleavage of structural proteins during the assembly of head of bacteriophage T4. *Nature* **227**: 680-685.

Lewis, N.G. and Yamamoto, E. (1990) Lignin: occurrence, biosynthesis and biodegradation. *Ann. Rev. Plant Physiol.* **41**: 455-561.

Li, G., Wanb, S., Zhoua, J., Yang, Z. and Qin, P. (2010) Leaf chlorophyll fluorescence, hyperspectral reflectance, pigments content, malondialdehyde and proline accumulation responses of castor bean (*Ricinus communis* L.) seedlings to salt stress levels. *Ind. Crops Prod.* **31**: 13-19.

Luthria, D. L., Mukhopadhyay, S. and Krizek, D. (2006) Content of total phenolics and phenolic acids in tomato (*Lycopersicon esculentum* Mill.) fruits as influenced by cultivar and solar UV radiation. *J. Food Com. Anal.* **19**: 771-777.

Maestri, D.M., Nepote, V., Lamarque, A. L. and Zygadlo, J.A. (2006) Natural products as antioxidants Phytochemistry: *Advances in Research* **2006**: 105-135.

Marchand, L. (2002) Cancer preventive effects of flavonoids: a review. *Biomed. Pharmacother* **56**: 296-301.

Maróstica, J .M. R., Leite, A. V. and Dragano, N. R. V. (2010) Supercritical fluid extraction and stabilization of phenolic compounds from natural sources – review (Supercritical extraction and stabilization of phenolic compounds). *The Open Chemical Engineering Journal* **4**: 51-60.

Mehr, Z. S., Hamideh, K., Bahabadi, S. E. and Sabbagh, S. K. (2012) Changes on proline, phenolic compounds and activity of antioxidant enzymes in *Anethum graveolens* L. under salt stress. *Intl. J. Agron. Plant. Prod.* **3** (S): 710-715.

Misra, N. and Gupta, A. K. (2005) Effect of salt stress on proline metabolism in two high yielding genotypes of green gram. *Plant Sci.* **169**: 331-339.

Misra, N., Gupta, A.K. and Dwivedi, U.N. (2006) Changes in free amino acids and stress protein synthesis in two genotypes of green gram under salt stress. *J. Plant Sci.* **1**: 56-66.

Mohamed, A.A. and Aly, A.A. (2008) Alternations of some secondary metabolites and enzymes activity by using exogenous antioxidant compound in onion plants grown under seawater salt stress. *American-Eurasian Journal of Scientific Research* **3**: 139-146.

Mostafazadeh-fard, B., Heidarpour, M., Aghakhani, Q.A. and Feizi, M. (2007) Effects of irrigation water salinity and leaching on soil chemical properties in an arid region. *Int. J. Agr. Biol.* **3**: 166-469.

Munns, R. and Tester, M. (2008) Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* **59**: 651-681**.**

Osawa, T. (1994) Novel natural antioxidants for utilization in food and biological systems. In: *"Postharvest Biochemistry of Plant Food-Materials in the Tropics"*, I. Uritani, V. V. Garcia and E. M. Mendoza (Ed.), pp. 241-251, Japan Scientific Societies Press, Tokyo, Japan.

Parida, A.K., Das, A.B., Sanada, Y. and Mohanty, P. (2004) Effects of salinity on biochemical components of the mangrove, *Aegiceras corniculatum*. *Aqua. Bot.* **80**: 77-87.

Petridis, A., Therios, I., Samouris, G. and Tananaki, C. (2012) Salinity-induced changes in phenolic compounds in leaves and roots of four olive cultivars (*Olea europaea* L.) and their relationship to antioxidant activity. *Enviromental and Experimental Botany* **79**: 37–43

Radi, A. A., Farghaly, F. A. and Hamada, A. M. (2013) Physiological and biochemical responses of salt-tolerant and salt-sensitive wheat and bean cultivars to salinity. *J. Biol. Earth Sci*. **3**(1): 72-88.

Radwan, S. A., El-Koly, A. S. and Sammour, R. H. (2013) Genetic variation among accessions of *Lathyrus inconspicuous* L. as revealed by SDS Polyacrylamide Gel Electrophoresis. *Acta Agriculturae Slovenic* **22**: 101 – 109.

Rady, M.M., Sadak, M. S.H., El-Bassiouny, H.M.S. and Abd El-Monem, A.A. (2011) Alleviation the adverse effects of salinity stress In sunflower cultivars using nicotinamide and α-Tocopherol. *Aust. J. Basic & Appl. Sci.* **5**(10): 342-355.

Sadak, M. S., Abdelhamid, T.M. and El-Saady, A.M. (2010) Physiological responses of faba bean plant to ascorbic acid grown under salinity stress. *Egypt. J. Agron.* **32**(1): 89-106.

Sánchez-Rodríguez, E., Moreno, D. A., Ferreres, F., Rubio-Wilhelmi, M. and Ruiz, J. M. (2011): Differential responses of five cherry tomato varieties to water stress: Changes on phenolic metabolites and related enzymes. *Phytochemistry* **72** (8): 723–729.

Sando, S. and Barrington, V. (2008) Heirloom Beans. "*Chronicle Books"*, p. 15.

Singleton, V. L., Orthofer, R. and Lamuela-Raventos, R.M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* **299**: 152-178.

Slimestad, R. and Verheul, M. J. (2005) Content of chalconaringenin and chlorogenic acid in cherry tomatoes is strongly reduced during post-harvest ripening. *J. Agric. Food Chem.* **53**: 7251-7256.

Snedecor, G.W. and Cochran, G.W. (1980) *"Statistical Methods"*, 7th ed., Iowa State, University Press, Ames, Iowa.

Sousek, J., Guédon, D., Adam, T., Bochorakova, H., Taborska, E., Valka, I. and Simanek, V. (1999) Alkaloids and organic acids content of eight *Fumaria* species. *Phytochem. Anal.* **10**: 6 -11.

Waseem, M., Athar, H.R. and Ashraf, M. (2006) Effect of salicylic acid applied through rooting medium on drought tolerance of wheat. *Pak. J. Bot.* **38**(4): 1127-1136.

Younis, M.F., Hasaneen, M.N.A., Ahmed, A.K. and El-Bialy, D.M.A. (2008): Plant growth, metabolism and adaptation in relation to stress condition XXI. Salinity and nitrogen rate effects on growth, photosynthesis, carbohydrate content and activities of antioxidant enzymes in *Lactuca sativa* transplents. *Aust. J. Crop Sci.* **2**: 83-95.

Zorb, C., Schamit, S., Need, A. and Karl, S. (2004) The biochemical reaction of maize (*Zea mays* L.) to salt stress is characterized by mitigation of symptoms and not by specific adaptation. *Plant Sci.* **167**: 91-100.

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تخفيف الاجهاد الملحي على ايض نباتي الشعير والفاصوليا باستخدام بعض الفينولات النباتية

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تهدف الدراسة إلى تخفيف اضرار الملوحة على نباتي الشعير والفاصوليا باستخدام بعض الفينولات النباتية الطبيعية من خلال المكونات الايضية. قسمت حبوب الشعير وبذور الفاصوليا الى خمس مجموعات الاولى نقعت في الماء والمجموعات من الثانية حتى الخامسة نقعت في المحاليل الفينولية المستخلصة من نباتات السبانخ والحمض والطماطم والكرنب لمدة 24 ساعة. زرعت حبوب الشعير وبذور الفاصوليا للمجموعات الخمس في اطباق بتري رويت بمحاليل ملحية متدرجة :
(00 mM NaCl, 50 mM NaCl, 100 mM NaCl and 200 mM NaCl) .

وأوضحت النتائج أن الاجهاد الملحي ادي الى نقص محتوي بادرات النباتين من السكريات والبروتينات الكلية وان نقع حبوب الشعير وبذور الفاصوليا في الفينولات النباتية قبل الزراعة ادى الى تحسين كمية السكريات والبروتينات تحت ظروف الاجهاد الملحي. وقد يعزى ذلك الى دور الفينولات النباتية في ضبط الاسموزية وحماية الانسجة الحية وزيادة الاصباغ النباتية وزيادة نشاط انزيمات مضادات الاكسدة مما يؤدي الى زيادة تحمل النباتات للملوحة. وأدت النتائج إلى ان تراكم الفينولات في بادرات كل من الشعير والفاصوليا يزداد بزيادة كل من الاجهاد الملحي والنقع المسبق في الفينولات النباتية. بينما الاجهاد الملحي ادي الى تراكم البرولين في الشعير والفاصوليا وان النقع قبل الزراعة في المحاليل الفينولية ادي الى نقص هذا التراكم مما يعتبر مؤشرا لزيادة تحمل النباتات للاجهاد الملحي. أظهر تحليل الحمل الكهربائي للبروتينات وجود تشابه بين كلا النباتين الناميين تحت ظروف الاجهاد الملحي في عدد كبير من الباندات. بينما ظهرت باندات جديدة نتيجة المعاملة ببعض الفينولات النباتية تحت ظروف الاجهاد الملحي. يستخلص من الدراسة ان معاملة حبوب الشعير وبذور الفاصوليا بالفينولات النباتية قبل الزراعة يلعب دورا محوريا في زيادة تحمل النباتات للاجهاد الملحي عن طريق تنشيط نظام ضد الاكسدة لكلا النباتين .