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Effect of chitosan on proteomics in *Lens culinaris* Medik plants grown under different levels of salinity *in vitro.*

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Abstract

The present study aimed to investigate molecular responses of *Lens culinaris* Medik. *(L. culinaris*) plantlets to salinity stress concomitant with investigating the role of exogenous bio-stimulants application strategy chitosan (CTS) in ameliorating the deleterious effect of salt stress. *In vitro*-produced plantlets of *L. culinaris* were treated with varying levels of NaCl (50, 100 and 150 mM) added to MS solid media and/or a long with CTS (0.1 mg L^{-1}). The molecular level, sodium dodecyl sulfate polyacrylamide gel electrophoresis technique (SDS-PAGE) for protein profile, was done. Analysing changes in SDS-PAGE protein banding patterns revealed a total number of 30 bands with molecular weights ranging from 15.2 KDa to 90.6 KDa. thirteen bands were observed as monomorphic, while seventeen bands were polymorphic, giving 43.33% polymorphism. Proteins banding was increased under salt stress compared to control induced as salt marker proteins.

Keywords: Chitosan; *Culinaris*; Salinity stress; Monomorphi; Polymorphism; Proteomics.

Introduction

Grain legumes are a good source of protein, accounting for 1740% of the total vegetable protein consumed by humans (*1*). In addition to their nutritional superiority, legumes have also been ascribed economic, cultural, physiological, and medicinal roles due to their possession of beneficial bioactive compounds (*2*). Many legume seeds are also good sources of iron, calcium, phosphorus, zinc, copper, and magnesium (*3*).They are particularly enriched in B vitamins

important for human nutrition, such as thiamine, riboflavin, niacin, folic acid and pantothenic acid, as well as vitamin C. Legumes are also valuable sources of tocopherols (*3*), proteins with essential amino acids, complexes carbohydrates, fiber, unsaturated fats, vitamins and essential minerals for human nutrition (*4*) . *Lens culinaris* Medik. is the most important food legume worldwide, a leading source of low cost proteins (*5*). They satisfy 34.6% of the total protein requirement worldwide and account for over 50% of all legumes consumed globally (*6*). Seeds of lentil have other valuable nutritional properties as fiber, minerals, vitamins, and low content of fat and sodium (*6*).

Chitosan is obtained *via* partial or total chitin deacetylation and can be classified as a copolymer of 2-amino-2-deoxy-β-d-glucopyranose (glucosamine) and 2-acetamide-2-deoxy-β-d-glucopyranose (N-acetyl-glucosamine). In general when the content of N-acetyl groups is >50 % is considered chitin, while for lower values is considered chitosan. It has nitrogen content of 6.80% or higher and is characterized by molecular weights between 1 × 105 and 5 × 105 Da (*7*). The discovery of chitosan is attributed to Rouget, who in 1859 found that when chitin was heated in an alkaline medium, a material that was soluble in organic acids was obtained (*8*). In 1894, Hoppe-Seyler called this material chitosan; however, only until 1950, its chemical structure was elucidated (*9*). As a biopolymer, chitosan has potential biomedical applications, since it is biocompatible and biodegradable. Due to its solubility in acidic aqueous medium, many applications at industrial level can be found for chitosan; its solubility is related to the degree of acetylation, molecular weight, and distribution of the acetyl and amino groups along the chain. Additionally, antimicrobial activity is attributed to chitosan when the amino groups are in cationic form, which means that antimicrobial activity of chitosan is higher at low pH (*10*). Chitosan has a broad-spectrum antimicrobial activity against both Gram-positive and gram-negative bacteria (*9*). Due to this property, chitosan is a natural antimicrobial agent with potential application in agriculture, food, biomedical and biotechnology fields (*8*). Chitosan has been used in the plants to confer resistance against abiotic stresses such as water deficit, salinity, heat stress and heavy metal toxicity (*11*).

Salinity stress involves changes in various physiological and metabolic processes, depending on severity and duration of the stress, plant genotypes, environmental factors and ultimately inhibits crop production. Initially salinity is known to represses plant growth manifested as osmotic stress, which is then followed by ion toxicity (*12*). In most of the cases, the negative effects of

salinity have been attributed to increase in sodium (Na^+) and chloride (Cl^-) ions in different plants hence these ions produce the critical conditions for plant survival by intercepting different plant mechanisms. Although both Na^+ and Cl^- are the major ions which produce many physiological disorders in plants, Cl^- is the most dangerous (*13*). During the initial phases of salinity stress, water absorption capacity of root systems decreases and water loss from leaves is accelerated due to osmotic stress of high salt accumulation in plants, and therefore salinity stress is also considered as hyperosmotic stress (*14*) .The outcome of these effects may cause membrane damage, nutrient imbalance, altered levels of growth regulators, enzymatic inhibition and metabolic dysfunction, including photosynthesis, protein and nucleic acid synthesis which ultimately leads to plant demise (*15*). Mechanisms of salt tolerance, not yet completely clear, can be explained to some extent by stress adaptation effectors that mediate ion homeostasis, osmolyte biosynthesis, toxic radical scavenging, water transport, and long distance response co-ordination (*16*). However, attempts to improve yield under stress conditions by plant improvement have been largely unsuccessful, primarily due to the mutagenic origin of the adaptive responses.

Salinity stress is one of the most deleterious a biotic stress factors that affect the growth, productivity and physiology of plants. Salinity imposes negative effect on the plant growth by decreasing leaf water potential, inducing morphological and physiological changes, production of ROS, increased osmotic stress, ion toxicity and by altering the biochemical processes (*14*) . Salinity tolerance involves not only a complex of responses at metabolic, physiological, and biochemical at the whole plant levels, but also at the molecular, cellular levels. Taking advantage of the latest advancements in the field of genomic, transcriptomic, proteomic, and metabolomic techniques, there is lack of the integration of information, and the combined approach is essential for the determination of the key pathways or processes controlling salinity tolerance. In addition, in spite of the significant progress in the understanding of plant stress responses, there is still a large gap in our knowledge at the molecular levels (*17*).

Proteomics, and in particular quantitative proteomics, is emerging as a powerful technique to be applied to the field of crop a biotic stress tolerance research; it has the potential to allow rapid identification and quantification of novel stress and tolerance-related proteins. Several common stress responsive proteins are expressed in response to various a biotic stresses in different plant

species. Some of them have been identified and characterized as salt-responsive proteins, which are either up regulated or down regulated by salinity stress (*18*) . Several salt-induced proteins have been identified in plant species (*19*). Protein band (26 kDa) associated with salt tolerance was noted in rice. Another study suggested that stress proteins could be used as important molecular markers for the improvement of salt tolerance using genetic engineering techniques (*20*). Similar synthesis of osmotin under salt stress was reported in many crops (*21*).There are many reports showing that the protein pattern changes are accompanied by the biological changes in the adaptation process, which makes the organism more fit in the altered environment (*22, 23*). Therefore this study was aimed to find out effect of chitosan on proteomics in *Lens culinaris* Medik plants grown under different levels of salinity *in vitro.*

Materials and Methods:

The seeds of Lentil (healthy and mature) used in this study were purchased from local market in Al Bayda, Libya. The plant was identified and authenticated as *Lens culinaris* Medik. (*L. culinaris*) by botanists at the Department of Botany (Silphium Herbarium), Faculty of Science, Omar Al-Mukhtar University, Al Bayda, Libya.

Media Preparation

Half basal (2.2) of Murashige and Skoog 1962 (MS) (*24*) constituents as shown in Table 1 supplemented with 30 g L^{-1} sucrose and 0.1 mg L^{-1} myo-inositol was used for *in vitro* seedlings germination. The pH media was adjusted to 5.8 before adding the solidifying agent and then autoclaved at 121ºC, 1.1 kg cm^{-2} pressure for 20 min. Autoclaved medium separately were poured into 350 ml jars (50 ml media/ jar) and stored at room temperature 25 ±2 ºC at least 3 days under completely darkness to examine contamination.

Surface sterilization and germination of *L. culinaris* seeds

The seeds were first washed with running tap water plus soap for 5 min and transferred to a clean and sterial flask. In a clean laminar flow hood, seeds were surface sterilization by 70% (v/v) ethanol for 2 min, and rinse with sterilize distilled water. Further, they are disinfected with 10% (v/v) of commercial clorox (5.25% Cl_2) containing two drops of a wetting agent tween 20 solution for 15 min and rinse three times with sterilize distilled water. In complete aseptic conditions sterilized equal number (three seeds) of sterilized

seeds were planted in each 50 ml germination medium, and incubated in a growth room 25 ±2 ºC with a daily 16hrs photoperiod (16/8 hours light /dark) under standard cool white fluorescent tubes, until germination seeds.

Regularly and after 15-25 days, jars were checked, photographed, the germination percentages were scored, and the best results were selected and old seedlings at the physiological age of 5-6 cm in length with 3-5 of develop leaves are subjectseds as a plant materials. Seedling re-culture on full (4.4) MS basal medium augmented with one of the four salinity level (0, 50, 100 and 150 mM) or with 0.1 mg L^{-1} chitosan (CTS, the potent concentration) alone and in combination. Different treatments were symbol led from M_1-M_8 as follows:

M_1= MS free NaCl and CTS (control)
M_2 = MS + 50 mM NaCl
M_3= MS + 100 mM NaCl
M_4= MS + 150 mM NaCl
M_5 = MS + 0.1 mg L^{-1}CTS
M_6 = MS + 50 mM NaCl + 0.1 mg L^{-1} CTS
M_7 = MS + 100 mM NaCl + 0.1 mg L^{-1} CTS
M_8 = MS + 150 mM NaCl + 0.1 mg L^{-1} CTS

Sub-cultivation of plants is carried out in saline medium and harvested at different times (3-10 days). Seedling removed from jars further washed with sterilized distilled water. Either of shoots or roots was excised carefully separated and, then stored in refrigerator at -20 ºC until uses.

Table 1. Composition of MS medium (Murashige and Skoog 1962).

Ingredients	Amount (mg/L)
Macronutrients	
NH_4NO_3	1650.00
KNO_3	1900.00
$CaCl_2.2H_2O$	440.00
$MgSO_4.7H_2O$	370.00
KH_2PO_4	170.00
Micronutrients	
KI	0.83
H_3BO_3	6.20
$MnSO_4.4H_2O$	22.30
$ZnSO_4.7H_2O$	8.60
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2$	0.025
Iron stock	
$FeSO_4.7H_2O$	27.80
$Na_2.EDTA.2H_2O$	37.30

Vitamins	
Myo-inositol	**100.00**
Nicotinic acid	**1.00**
Pyridoxine HCl	**1.00**
Thiamine HCl	**10.00**
Glycine	**2.00**
Sucrose (g)	**30.00**
Agar (g)	**8.00**

Molecular Analyses

SDS-protein electrophoresis

Sodium dodocylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to study the protein profiles of *L. culinaris* under control and the chitosan investments. Protein fractionation was performed following Laemmli (*25*) as modified by Studier protocol (*26*). Gel was photographed using a 35 mm colour film (100 ASA) and scanned with Bio-Rad Video densitometer Model 620. Software data analysis for Bio-Rad Model 620 densitometer and IBM compatible personal computer 165-2072 were used. (Plant Biotechnology Department, Genetic Engineering and Biotechnology Division, National Research Centre, Dokki, Cairo, Egypt).

Result

The protein banding patterns of leaves of *L. culinaris* seedling as revealed by electrophoretic separation using SDS-PAGE technique was illustrated in Figure 1and Table 3. In the present study modifications were assessed (under salt stress, chitosan application and their combination) as *de novo* bands, disappearance of some and selective expression in others. Total number of bands was 30 with molecular weights ranging from 15.2KDa to 90.6 KDa. Demonstrative analysis for the presence and absence of bands were assessed with 1 and 0, respectively.

Demonstrating the protein banding patterns, type, number and polymorphism calculations in Tables 2- 3 clearly point to that *L. culinaris* leaves under all treatments and regardless of age comprised thirteen bands as common bands (monomorphic) their molecular weights were 90.9, 73.3, 62.7, 56.2, 55.2, 53.5, 50.8, 45.3, 41.8, 39.6, 35.0, 28.1 and 21.8 KDa represented 43.33% polymorphism.

On the other hand, scanning the gel explore twelfths non –unique protein bands represent 40% polymorphism. Meanwhile, four bands were assessed as unique bands having molecular weights 80.5, 67.2, 48.6 and 18.1 KDa represent 13.3% polymorphism.

In the present study, treatment received CTS alone at the second age induced the synthesis of one new protein band considered as unique having the molecular weight of 80.5 kDa compared with the control. During the first age, control of leaves (M_1) was discriminated by the absence of unique band with molecular weight 67.2 KDa. Also, MS medium supplemented with 150 mM NaCl and 0.1 mg L^{-1} chitosan (M_8) were recognized by two negative unique protein bands with molecular weights 48.6 KDa and 18.1 KDa.

Scanning the resulted bands point to that 50 and 100 mM NaCl in combination with 0.1 mg L^{-1}CTS (M_6, M_7) lead to disappearance of protein with molecular weight 82.2 KDa referred to control. In addition, during first age protein with 33.0 KDa molecular weight disappeared from control and all other treatments, while expressed during the second age with the control and the lost salt does only.

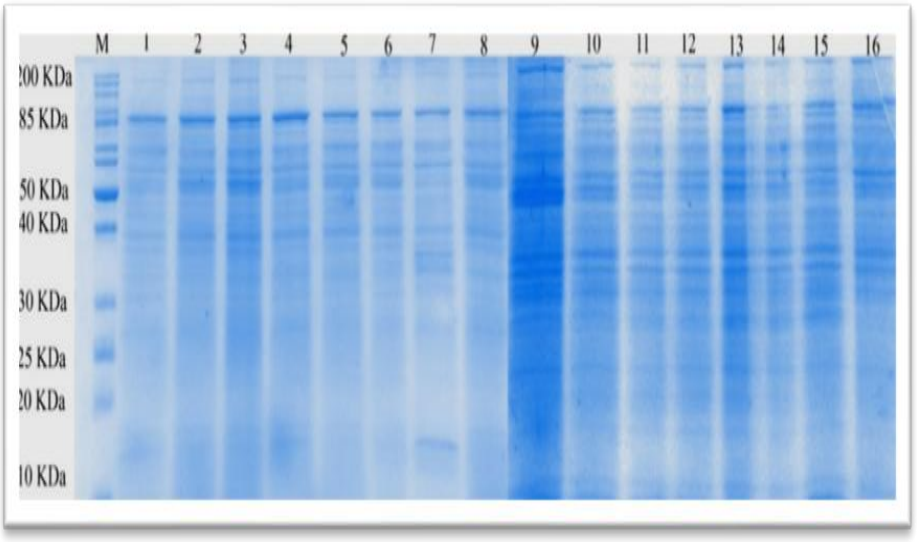

Figure 1: Electrophoretic banding patterns separated by SDS of leaves of *in vitro* of *L. culinaris* germinated seedling cultured in free MS and /or MS invested with different concentrations of NaCl alone or along with CTS, harvested three and ten days later**.**

Table 2: Number and types of bands as well as the percentage of the total polymorphism generated in protein markers of leaves of *in vitro* of *L. culinaris* germinated seedling cultured in free MS and /or MS invested with different concentrations of NaCl alone or along with CTS, harvested three and ten days later.

Protein markers					
Type of band	**Total bands**	**Monomorphic bands**	**Polymorphic bands**		**Polymorphic (%)**
			Unique bands	**Non unique bands**	
No. of bands	30	13	4	13	56.66

Table 3. The presence (1) and absence (0) of bands of leaves proteins electrophoretic banding patterns and molecular weights (MW) of SDS proteins in *in vitro L. culinaris* germinated seedling cultured on free MS and or/ MS invested with different salt levels and CTS and harvested 3 and 10 -days after application.

Harvesting time (day)	**3 days**									**10 days**							
Band number	**MW**	**M_1**	**M_2**	**M_3**	**M_4**	**M_5**	**M_6**	**M_7**	**M_8**	**M_1**	**M_2**	**M_3**	**M_4**	**M_5**	**M_6**	**M_7**	**M_8**
1	**90.6**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	**82.2**	0	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1
3	**80.5**	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
4	**77.6**	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
5	**73.3**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	**71.4**	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
7	**68.5**	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
8	**67.2**	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	**65.1**	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
10	**62.7**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	**56.2**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	**55.2**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	**53.5**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	**50.8**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	**48.6**	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
16	**45.3**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	**41.8**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	**40.2**	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0
19	**39.6**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	**35.0**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
21	**33.0**	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
22	**32.5**	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
23	**30.8**	0	0	1	1	1	1	1	1	0	1	1	0	0	0	0	0
24	**28.1**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
25	**25.3**	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
26	**23.8**	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
27	**21.8**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
28	**18.1**	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
29	**17.1**	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
30	**15.2**	0	0	0	0	1	0	0	1	1	1	1	1	1	1	1	1
Total bands		**18**	**22**	**23**	**21**	**23**	**20**	**21**	**21**	**25**	**23**	**22**	**22**	**23**	**22**	**22**	**22**

Where:

M_1= MS free CTS and NaCl (control)
M_2 = MS + 50 mM NaCl
M_3= MS + 100 mM NaCl
M_4= MS + 150 mM NaCl
M_5 = MS + 0.1 mg L^{-1} CTS
M_6 = MS + 50 mM NaCl + 0.1 mg L^{-1} CTS
M_7 = MS + 100 mM NaCl + 0.1 mg L^{-1} CTS
M_8 = MS + 150 mM NaCl + 0.1 mg L^{-1} CTS

Discussion

Overall, application of CTS was helpful in improving salinity tolerance in the lentil seedling and its application may stimulate the differences defense mechanisms of plants against salt toxicity. Suggested strategy is that external bio-stimulants can alleviate salt toxicity. In an attempt to understand how cellular salt tolerance (physiological ,biochemical and molecular) mechanisms are integrated and coordinated in *L. culinaris* plantlets and to evaluate the applied strategy successfulness *in vitro* in adapting plantlets to salt tolerance , different wide parameters involved in salt response were applied. Salt stress causes alterations in plant metabolism, including a reduced water potential, ion imbalances because it disturbs the uptake and translocation of mineral nutrients, oxidative stress and hormonal imbalance toxicity and sometimes severe salt stress may even threaten survival (*27*). In addition, salt stress can trigger various interacting events, including inhibition of enzymatic activities in metabolic pathways, and decreased carbon-use efficiency and decomposition of protein and membrane structures (*23*).The mechanisms by which plants defend them against saline stress are indeed many fold; many are still unclear and they may vary according to the ontogenetic stage.

On the other hand, the mechanism of action of CTS has been an attractive target for many researchers but it is still far from final solution. Considering the high variability of the physiological effects of CTS, it is believed that more than one molecular mechanism of their action exists. Two main aspects of the primary mechanism have to be considered: 1) the effects of CTS on membrane properties and 2) the effects of CTS on the biosynthesis of different enzymes *via* their effects on gene expressions. Plantlets resulted from tissue culture techniques always were very delicate and must be carefully handle for laps of time to ensure successful regeneration protocol. Here the study took the challenge that subculture the resulted plantlets aged 5 weeks into MS only or MS invested with CTS alone and /or with either of the four NaCl levels (at 0, 50, 100 or 150 mM) can cope such harsh environments giving future hope to tolerate such sensitive cultivar against one of the most important challenge environmental clue.

The present investigation reveals that 5-weeks old *in vitro L. culinaris* plantlets sub cultured on MS and challenged with NaCl in comparison with those

sub cultured on free MS, suffer from salt stress illness manifested as retardation in many of metabolic activities, concomitant with retardation in oxidative stress scavenging systems activities, imbalance in endogenous hormones and ion accumulation. This is corroborated with previous reports describe *L. culinaris* plants as relatively sensitive to salt stress (*12*). However, this reduction in most *in vitro* seedlings parameters was alleviated in MS medium invested with CTS alone which was found to improve the cultured seedlings performance as manifested from the marvelous increment in estimated same previous parameters. Therefore, the study hypothesis was that CTS could alleviate salt stress negative impact of *in vitro L. culinaris* regenerated seedlings *via* different collaborated strategies and enhance seedlings tolerance. The collected promise data points to that CTS successfully tolerate *in vitro L. culinaris* regenerated seedlings to face harsh salt condition. To rescue deleterious effects of plant salt stress and enhance plant stress tolerance, exogenous CTS application was considered a powerful strategy manifested as change in plants gene expression and protein accumulation. In an attempt to understand the molecular basis of salt tolerance *in vitro L. culinaris* seedlings, SDS-PAGE was used to identify changes in protein banding patterns. Moreover, many proteins undergo post-translational modification, such as removal of certain parts, phosphorylation, and glycosylation, which is extremely important for protein activities and subcellular localization. Therefore, it is necessary to study the salt stress responses at the protein level (*18*).

Analyzing protein gel, three types of modifications were observed in the protein patterns some bands were disappeared, other proteins were selectively increased, and synthesis of new sets of protein was induced. Some of these responses were engaged with CTS along with salt treatments, while others were induced by either CTS or salt stress. In plants, new proteins synthesized in response to interaction between environmental stress and CTS substances application has been reported as stress protein. Many of these proteins were suggested to alleviate and protect cells against the adverse effect of stress condition. Changes in protein synthesis under salt stress and exogenous application of CST treatments may be due to modifications in the efficiency of mRNA function or post translation or to changing at transcription level (*28*). The raise of seedlings protein content can be due to induction of specific proteins involved in stress tolerance/response. Beside their specific functions, proteins which are accumulated in the plants by stress exposure may provide a storage form of nitrogen that is reutilized when stress is over and probably play a role in osmotic adjustment (*29*).

Results presented in this study, showed that *in vitro L. culinaris* seedlings sub cultured in MS salinized with sodium chloride stress levels (50, 100 mM) compared to free MS and harvested three days after, induced the formation of four proteins considered *de novo* proteins (Tables 2,3 and Fig.1).Two high molecular weight proteins (82.2 and 77.6 KDa) and low molecular weight one (32.5 KDa) were considered as unique proteins associated with NaCl broad, fast and quick response to prevent any structural cell damage. It is possible, that, this process of cellular adaptation of the seedlings to salinity stress, regulated by specific alterations in gene expression of NaCl adapted cells resulting into the synthesis of such unique proteins. In the same line, Shobbar *et al*. (*30*) stated that a protein band with the molecular mass of 66.2 kDa in FL478 (rice cultivar) were recorded under salt stress suggests a probable role of this protein in salt tolerance.

Salinity induced protein with molecular weight 26 kDa was reported in barley roots (*22*) , rice shoots and cultured cells (*31*) and in tomato (*32*). Previous results supported the present study in which protein with molecular weight 25.3 ,23.8,17.1 and 15.2 kDa were absent after three days but after that expressed in all treatment may be considered as constitutive and age dependent increased under salt stress as protective ,because this protein had critical function in plant development under biotic and a biotic stress. It is widely known that many responses may be just transient responses against environmental stress and that many of their related genes are common to several types of stresses not specific (*33*). Plants have different chitosan sensitivities, depending on endogenous or exogenous factors, such as organ type, environment, and growth stage. Chitosan 80.5 KDa protein was investigated and identified by Dutta (*34*) and involved in ribosome biogenesis which was considered the major function known to be associated with the nucleolus (*31*), although recent work suggest that this nuclear compartment might be involved in other cellular processes (*33*). In the present study unique CTS protein with molecular weight 80.5 KDa was expressed in CTS treatment alone ten days later and so the study can suggest that may be it need certain concentration to be expressed or it was age dependent.

Conclusion:

In recent decades, exogenous protectant such as osmoprotectants as proine, plant hormone as brassinosterioids, bio-stimulants as chitosan, antioxidants as ascorbic acid, signaling molecules as nitric oxide, polyamines as putrescine and trace elements as selenium have been found effective in mitigating the salt induced damage in plant. These protectants showed the capacity to enhance the plant growth, yield as well as stress tolerance under salinity. Many compounds are

being used to cope with the toxic effects of salinity among them chitosan treatments successfully overcame the toxicity generated by NaCl-stress and almost levelled the values with the control. Mechanism of chitosan in plants has not been fully understood yet. However, there are many reports suggesting chitosan elicited a number of defence responses in the plants. Chitin-specific receptors are present in plant cell membranes which are known to elicit defence responses. When treated with chitin-based treatment, plants activate their defence mechanism since they mimic compounds related to chitin-containing organism.

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تأثير الشيتوزان على (بروتينات الاجهاد) في نباتات العدس (*Lens culinaris* Medik) النامية تحت مستويات مختلفة من الملوحة في المختبر

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إستكشاف برنامج لإعادة إنتاج أخلاف للعدس صنف كولينارس تحت الدراسة في المعمل هو مطلب أولى للوصول إلى جيل جديد موسوم وراثيا بالصفات الجديدة المرجوة. وقد أظهرت العديد من الدراسات أن المنشط الحيوي الشيتوزان (CTS) له أدوار عديدة على المستوى الخلوى أهمها الدور الإيجابى فى الإسراع بإنقسام وإستطاله وتكشف الخلايا وأيضا علي تخفيف الأثر السلبي للإجهاد الملحي وغيره في النباتات. تهدف الدراسة الحالية لدراسة الإستجابات الجزيئية في شتلات العدس المجهده ، يصاحب ذلك تقييم إستراتيجية الإستخدام الخارجي للشيتوزان بأنه يؤدى لتخفيف التأثير الضار للإجهاد الملحي.

شتلات العدس والتي عمرها ثلاثة أسابيع وكانت منماه على نصف القوة للبيئة موراشيج وسكوج (خالية من أي إضافات هرمونية) تم نقلها وإعادة زراعتها مرة أخرى على بيئة موراشيج وسكوج بها مستويات مختلفة من كلوريد الصوديوم (150،100,50 مليمول) والشيتوزان (0.1 مجم / لتر) مفرده أو مجتمعة فى تجربة قصيرة وحصدت العينات للتحليل بعد ثلاثة وعشرة أيام من التعرض للمعاملة .وقد تم التحليل على المستوى الجزيئي تم إستخدام طريقة التفريد الكهربي (PAGE) لتحديد البصمات الوراثية للبروتين للتحقيق في أي خلافات جزيئية بين شتلات العدس في ظل ظروف الدراسة. لقد كشف التفريد الكهربي (SDS-PAGE) للبروتينات في الأوراق علي إجمالي 30 نوع من البروتينات المختلفة والتي تتراوح أوزانها الجزيئية بين 15.2 إلى 90.6 كيلو دالتون. وقد أظهر التحليل ثلاثة عشر منها متماثلة، في حين كانت سبعة عشر منها غير متماثلة، مسجلة 43.33٪ عدم تماثل. زادت أنواع البروتنيات تحت الإجهاد الملحي مقارنته بالكنترول وأعتبرتخليق تلك البروتينات علامة للملوحة. فى حين وجد أن CTS (0.1 مجم / لتر) إلى بيئات موراشيج وسكوج و حده أو جنبا إلى جنب مع مستويات الملح المختلفة وخاصة مع أقل تركيز مستخدم من الملح قد خفف من التأثير الضار للإجهاد الملحي بشكل ملحوظ من خلال تعزيز الكشف عن بروتين الوزن الجزيئي له 80.5كيلو دالتون كعلامة مع إستخدام CTS, في حين إستخدام الشيتوزان مع كلوريد الصوديوم 50 ،150،100 مليمول أدت لإختفاء أنواع من البروتينات ذات أوزان جزيئية مختلفة مقارنة بلكنترول.

الكلمات المفتاحية: بروتينات الاجهاد , نبات العدس , الملوحة