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CHANGES IN ENDOGENOUS POLYAMINES AND PROTEIN FRACTIONS IN SALT STRESSED WHEAT PLANT BY PUTRESCINE OR SPERMINE TREATMENTS

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ABSTRACT

A pot experiment was carried out to study the effect of polyamines pretreatments, putrescine (4&8 mM) or spermine (6 & 10 mM), on the content of endogenous polyamines and protein fractions in wheat plants grown under salinity stress (0.0, 8000 and 10000 ppm NaCl). NaCl stress produced new protein bands, with molecular masses (mM) of 80, 40 and 16 KD. Also new bands appears in plants grown under putrescine or spermine treatments. The common new bands which appeared with polyamines application were Mm 80, 45, 40, 24, 16 and 14 KD, compared to their control. Content of putrescine decreased gradually with increasing NaCl level in irrigation water while, spermine and spermidine contents were generally increased under salinity stress.

INTRODUCTION

Biotic stresses such as soil/water salinity lead to cellular water deficit and ion toxicity in all cells (Greenway and Munns, 1980; Munns 2002). Plants have developed different strategies to withstand such stress. These include mechanisms which facilitate ion exclusion/sequestration (Yeo and Flowers, 1982), accumulation of compatible solutes for turgor maintenance and detoxification of free radicals (Bohnert and Jensen, 1996).

Water stress also induces accumulation of a large number of stress proteins variously described as LEA (late embryogenesis abundant protein), dehydrins, osmotine and others (Baker *et al*., 1988; Bray, 1997; Dure, 1992). The differences in polyamines (putrescine, spermidine and spermine) response under salt-stress have been reported among and within species. For example, according to Prakash and Prathapasenan (1988), endogenous levels of polyamines decreased in rice seedlings under NaCl- stress, whereas Basu *et al.,* (1988) reported that salinity resulted in accumulation of these compounds in the same material. Krishnamurthy and Bhagwat (1989) reported that salt-tolerant rice cultivars drastically accumulated high level of spermidine and spermine resulting in an enhanced level of total polyamines, with a relative decrease in putrescine content.While, the contrary was observed in sensitive cultivars.

MATERIALS AND METHODS

A pot experiment was carried out during the growth period, November (2005) to April (2006) to study the effect of salinity application as well as polyamine pretreatments on the content of endogenous polyamines and on the protein bands of wheat plants. The used grains of wheat plant (*Triticum Aestivum* L.) sakha 93 were obtained from the Department of Wheat Research, Agriculture Research Center, Giza, Egypt.Three levels of water salinity of zero, 8000 and 10000 ppm NaCl were used for irrigation of the plants of each treatment. Mature grains of wheat (sakha 93 cultivar) were soaked for 8 hr's at 20 + 1°C in distill water (control) or in putrescine at the concentrations, 4 and 8 mM and or in spermine concentrations at 6 and 10 mM.

Method of planting:-

Grains of wheat cultivar were planted at depth of 4-5 cm from soil surface. The pots were irrigated with tap water until the complete germination (7 days) then the plants were thinned to identical 8 plants/ pot. After wards the plants were irrigated every 15 days either with tap water (in the control treatment) or with one of the different concentrations of saline water to keep the soil at the level of 70% of the field capacity. Saline soil was washed with tap water from time to time to decrease salt accumulation in the soil. The plants were harvested at 150 –days –old plants.

Protein Fractionation

Sodium dodecyl sulfate polyacrylamide slab gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970), as modified by Studier (1973) for the fractionation of total protein in grains of wheat plants.

Estimation of polyamines:

Putrescine, spermidine and spermine were determined in shoot of 75-days-old plants (at heading stage) treated with 4 & 8 mM putrescine or 6 & 10mM spermine of plants grown under 8000 and 10000 ppm NaCl as well as control one. Polyamines, (putrescine, spermidine and spermine) were extracted and determined according to Ayesh *et al*. (1995); Mietz and Karmas (1997). The results were expressed as µg/g dry weight.

RESULTS AND DESICUSSION

Effect of salinity and polyamines on protein fractions:-

Data presented in Table 1 and photo (1&2) show the results of sodium dodecyl sulphate polyacrylamide gel electrophoresis on wheat grain proteins as affected by different levels of salinity(zero, 8000, and 10000 ppm NaCl) as well as polyamines application, 4 & 8 mM putrescine and 6 & 10 mM spermine.

Protein fractions (molecular mass KD) were increased due to salinity stress, control plant contained thirteen bands only meanwhile plants grown under 8000 or 10000 ppm NaCl treatments contained fourteen and fifteen bands, respectively. It is observed from Table 1 that the use of NaCl, either 8000 or 10000 ppm, produced new bands, with molecular masses (mM) of 80, 40 and 16 KD as compared to the control bands (without salinity). It is also observed that the band of molecular mass 74 (high Mm) was absent by the action of salinity.

Concerning the application of polyamines, putrescine or spermine, on protein bands, it is observed from Table 1 that there were a new bands appears in treated plants. The common new bands which appeared with polyamines application were Mm 80, 45, 40, 24, 16 and 14 KD, compared to their control (Salinized without polyamines). It is observed from Table 1 that polyamines application increased protein fractions either under control or with salinity treatments. From the previous results it could be observed that the use of NaCl produced new bands may be due to induce the synthesis of stress proteins. The appearance of new protein bands and the disappearance of others may be a response to salt stress, the new protein bands ranged from 80 to 16 KD.

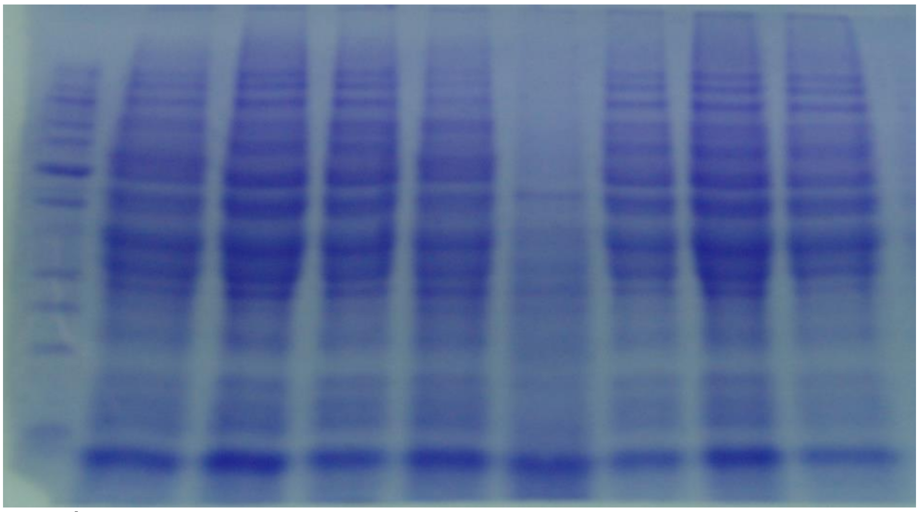
Table 1 : SDS-PAGE of total protein bands in grains of wheat plant as affected by studied treatments:

Mol. Wt of protein	Polyamines														
	Zero			(4 mM put.)			(8 mM put.)			(6 mM spm.)			(10 mM spm.)		
	NaCl ppm			NaCl ppm			NaCl ppm			NaCl ppm			NaCl ppm		
	Zero	8000	10000	Zero	8000	10000	Zero	8000	10000	Zero	8000	10000	Zero	8000	10000
194	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
155	+	+	+	+	-	+	+	+	+	+..	+	+	+	+	+
116	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
96	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+
80	-	+	+	-	-	+	+	+	-	+	+	+	+	+	+
74	+	-	-	-	-	-	-	-	+	+	+	+	+	+	-
66	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
50	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+
45	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
40	-	-	+	-	+	-	-	-	+	+	+	-	+	+	-
35	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+
30	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+
27	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-
24	-	-	-	-	+	-	-	-	+	+	-	+	+	+	+
20	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+
16	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	-	-	-	+	-	+	-	-	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

In this respect, Ramagopal (1988) studied the responses of barley genotypes, CM 72 (a salt-tolerant cultivar) and prato (a salt-sensitive cultivar), toward salinity stress. He demonstrated that salinity induced unique proteins in roots and shoots of CM 72 as well as prato.

Chourey *et al*. (2003) reported that germination and subsequent hydroponics growth of rice plants under salt stress (100 mM NaCl) triggered an accumulation of six major stress proteins. In this study they showed that several salinity stress-induced LEA proteins accumulated during the salt stress triggered growth arrest of young rice seedlings. Rice also accumulates several stress proteins, such as the RAB family (Mundy and Chua, 1988), the Dehydrine family and the LEA family of proteins (Mundy and Chua 1988; Moons *et al.,* 1995) and their transcripts during exposure to salts stress. The LEA proteins have been suggested to play a major protective role during desiccation/salinity stress in rice plants (Moons *et al*., 1995). In particular, the group 3LEA proteins possess tandem repeats of an 11 amino acids motif that can form an amphiphillic α-helix structure which readily binds ions (Dure *et al*., 1989).

M 1 2 3 4 5 6 7 8



M: Marker

Photo 1. The effect of studied salinity and polyamines treatments on wheat grains proteins.

1-Zero ppm NaCl + zero Polyamine. 2- 8000 ppm NaCl + zero Polyamine.
3- 10000 ppm NaCl + zero Polyamine. 4- Zero ppm NaCl + 4 mM Putrescine
5- 8000 ppm NaCl + 4 mM Putrescine. 6- 10000 ppm NaCl + 4 mM Putrescine.
7- Zero ppm NaCl + 8 mM Putrescine. 8- 8000 ppm NaCl + 8 mM Putrescine.

Concerning polyamines effect on the electrophoretic pattern of protein in wheat grains, putrescine or spermine treatments induced disappearance of certain bands and the appearance of new ones as compared with the untreated plants. In this respect, Mizzrahi *et al*. (1989) concluded that, polyamines are important cellular constituents to specific regulatory proteins. They provide a possible mechanism for the formation of polyamine-protein complexes. Kuznetsov and Shevyakova (1997) stated that, polyamines could change the stability and substrate specificity of protein kinase/phosphatase systems to modify the properties of polypeptide and acting as substrates for phosphorylation and dephosphorylative enzymes and affect the stability of protein molecules in plants.

Also, Change and Kang (1999) stated that, polyamines stimulate the phosphorylation of 17, 26, 30 and 35 KDa of petioles of *Rannuculus* plant. El-Bassiouny (2004) revealed that, the application of putrescine on pea plants induced the synthesis of new proteins at Mwts. 157, 111, 55, 41 and 29 KDa.

In this connection, El-Bassiouny *et al*. (2008) revaled that protein electrophoratic pattern of the treated wheat plants at 75 days old showed that, arginine or putrescine application induced the appearance of 5 new protein bands at molecular weights 222, 131, 93, 50 and 14 KDa.

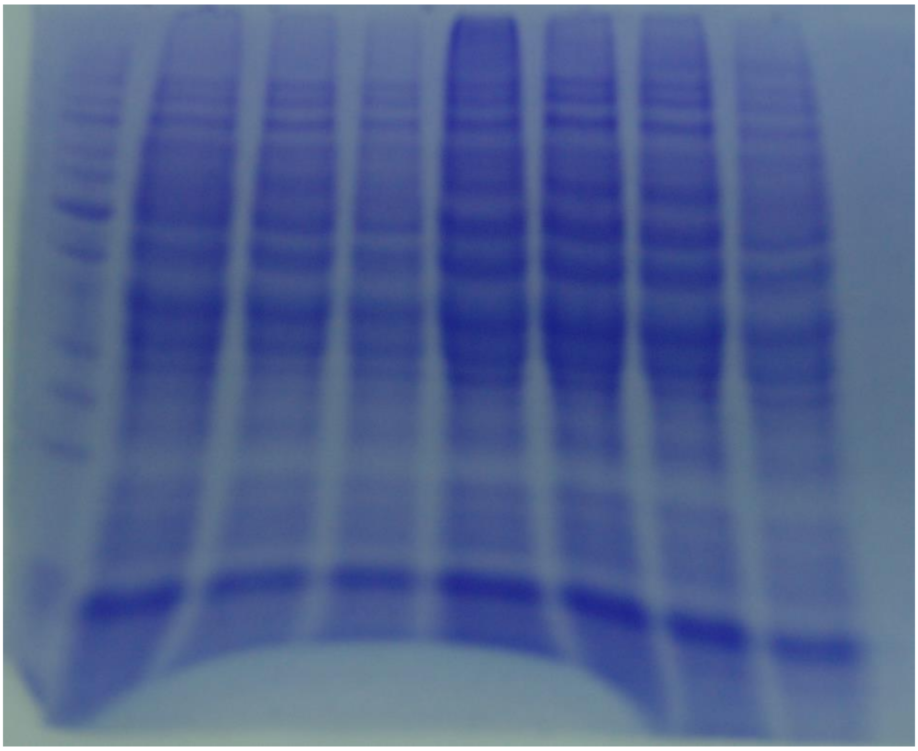


Photo 2. The effect of studied salinity and polyamines treatments on wheat grains proteins.

9- 10000 ppm NaCl + 8 mM putrescine. 10- zero ppm NaCl + 6 mM spermine.
11- 8000 ppm NaCl + 6 mM spermine. 12- 1000 ppm NaCl + 6 mM spermine.
13- Zero ppm NaCl + 10 mM spermine. 14- 8000 ppm NaCl + 10 mM spermine.
15- 10000 ppm NaCl + 10 mM spermine.

Effect of salinity and polyamines application on endogenous content of polyamines:-

Data presented in Table 2 show the effect of different salinity levels (zero, 8000 and 10000 ppm NaCl) as well as various level of polyamines (4 & 8 mM putrescine, 6 & 10 mM spermine) on the endogenous levels of polyamines in wheat shoots at heading stage, 75-days-old plants.

1- Effect of salinity:-

It is observed that the content of putrescine decreased gradually with increasing NaCl level in irrigation water. Spermine and spermidine contents were generally increased under salinity stress compared to control plant (without salinity). In general, the amount of putrescine was more than spermine and spermidine in shoot system of wheat plants.

2- Effect of polyamines

Presoaking grains in 4mM and 8 mM putrescine before cultivation leads to increase of putrescine levels in shoots of wheat plant under control conditions (unsalinzed plants). While, presoaking grains in 6 mM or 10mM spermine showed a little decrease of spermine content of shoots. In salinized plants, putrescine application leads to rise of putrescine contents in shoots as compared to salinized untreated plants, while the concentrations of spermine and spermidine showed a little decrease. These results were true in plants grown under 8000 ppm NaCl. Plants grown under 10000 ppm NaCl showed an increase in spermine content, as compared to salinized untreated plants.

In respect to spermine treatments (6mM and 10mM), under salinity stress all studied polyamines were decreased as a result of spermine application in shoots of wheat plants grown under 8000 ppm NaCl. Plants grown under 10000 ppm NaCl had the same trend except the spermine concentration of plants grown under spermine treatments, 6 and 10 mM .

The above results showed that putrescine concentration in shoot sample of wheat plant was decreased with increasing salinity levels, while spermine and spermidine were increased under stress condition. Polyamine treatments lead to an increase of putrescine levels, while, decreased spermine and spermidine levels in most cases as compared to their control plants were showed.

Table 2 : Concentration of Endogenous Polyamines (µ g /g dry wt.) in Shoots of Wheat Plant Under Different NaCl Levels and Polyamines Treatments

NaCl (ppm) \ Polyamines (mM)		Zero	Putrescine (mM)		Spermine (mM)	
			4	**8**	**6**	**10**
Zero	Put.	0.248	2.60	9.24	0.236	0.170
	Spm.	0.018	N.D	0.019	0.018	0.012
	Spd.	0.018	0.009	0.008	0.006	0.014
8000	Put.	0.164	0.600	2.34	0.130	0.016
	Spm.	0.033	0.021	0.014	0.012	N.D
	Spd.	0.011	0.005	0.005	0.008	0.009
10000	Put.	0.155	0.309	2.34	0.042	0.005
	Spm.	N.D	0.012	0.015	0.023	0.049
	Spd.	0.021	0.004	0.007	0.013	0.024

These results are in harmony with those obtained by (Krishnamurthy and Bhagwat, 1989; Kakkar *et al.,* 2000). External stress can either result in an increase or decrease in cellular polyamines, depending upon the type of stress, the plant species and the time of stress application (Kandpal and Appajraso, 1985). Salt stress was found to modify polyamine distribution between seedling organs indicating that polyamine responses to salt stress were functional in whole plants (Ghoulam and Fares, 2001). A general response by different plant species to salinity in relation to polyamine production is that polyamine levels change with salinity, in most cases, putrescine decreased while spermine and/or spermidine increased.

El-Shintinawy (2000) indicated that salinity greatly enhanced the accumulation of spermine and spermidine contents associated with a decrease in putrescine content in wheat cultivars. Accordingly, our result indicated general trends whereby salinity decreased putrescine levels while increased spermine and/or spermidine. This reflects that the pool of putrescine is deviated to spermidine and spermine synthesis. According to Aziz *et al*. (1998), a decrease in putrescine could be explained because under salt stress putrescine catabolism (via diamine oxidase) can contribute to proline (a compatible osmolyte) accumulation. Exogenous application of polyamines has been shown to protect against various stress conditions such as cold, wilting, pollution and salinity (Sun *et al*., 2002).

Presoaking application of putrescine stimulates the endogenous putrescine content under NaCl salinity is in accordance with the results obtained by Prakash and Prathapasenan (1988). It has been hypothesized that the increase of endogeneous putrescine may be play specific protective roles in plants adapted to extreme environment (Kuehn *et al*., 1990) as well as application of putrescine appeared to counteract the salt effect of many plants.

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التغيرات فى محتوى نبات القمح المجهد ملحياً من عديدات الأمين والحزم البروتينية إستجابة للمعاملة بالبوتريسين والإسبرمين

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أجريت تجربة أصص على نبات القمح المجهد ملحياً لمعرفة مدى تأثير المعاملة بالبوتريسين والأسبرمين على المحتوى الداخلى من عديدات الأمين وكذلك الحزم البروتينية المختلفة. لوحظ تغيير في عدد البروتينات الناتجة عن الفصل الكهربي للبروتين نتيجة المعاملة بالملوحة . أدي استخدام كلوريد الصوديوم (8000 أو 10000 جزء من المليون) إلي ظهور أنواع جديدة من البروتين ذات الأوزان الجزيئية 80 ، 40 ، 16 كيلو دالتون . أدت المعاملة بالبتروسين أو الاسبرمين أيضاً إلي ظهور حزم من البروتين وذلك في وجود الملح أو عدم وجوده . كانت البروتينات الجديدة ذات أوزان جزيئية مختلفة وهي 80 ، 45 ، 40 ، 24 ، 16 ، 14 كيلو دالتون .

أشارت النتائج إلي وجود نقص ملحوظ وتدريجي في محتوي نبات القمح من البتروسين مع زيادة مستوي كلوريد الصوديوم في التربة. من ناحية أخري كانت هناك زيادة كبيرة في محتوي النباتات من الاسبرمين والاسبرميدين تحت تأثير الملوحة بالمقارنة بالنباتات من مجموعة المقارنة . كان لمعاملات عديدات الأمين تأثير عكسي حيث أدي وجودها إلي زيادة ملحوظة في البتروسين وأيضاً نقص ملحوظ في كلاً من الاسبرمين والاسبرميدين في معظم الحالات بالمقارنة بالبيانات الواقعة تحت تأثير الملح وغير المعاملة بعديدات الأمين .

قام بتحكيم البحث

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