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PROTEIN AND AMINO ACID CONTENTS IN SOME RANGE PLANTS AS AFFECTED BY DIFFERENT STRESS CONDITIONS

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ABSTRACT

The present work aimed to study the effect of habitat conditions on the total crude protein, total amino acids and protein electrophoresis in some range species growing naturally in wadi El-Gafra.

Four species were investigated from two different habitats viz., up stream portion of Wadi El-Gafra, near the high way at 63km west of Suez and Mid stream portion of the same Wadi just N-NW El-Asher Min Ramadan City Egypt. These species included *Panicum turgidum*, Forssk; *Lasiurus hirsutus,* Forssk; *Tamarix mannifera*, Ehrenb. And *Nitraria retusa*, Forssk.

The results indicated that there were variations in the concentrations of total crude protein and protein amino acid of plants in response to habitat conditions, i.e., the species of xeric habitat (*Panicum turgidum* and *Lasiurus hirsutus*) accumulated cyclic amino acids less than the other species which collected from saline habitat (*Tamarix mannifera* and *Nitraria retusa*). Also the studied halophytes accumulated proteins with low molecular weight than xerophytes species.

INTRODUCTION

Desert vegetation thrives under unique conditions of environmental stresses. Soil-and/or atmospheric drought are generally persistent factors in such habitat. Besides, high thermal regimes during daytime and/or night/day temperature extremes add to drought stress imposed on such vegetation. In certain locations, where physiographic conditions call for, soil salinization may replace soil drought. Accordingly, the atmosphere-plant-soil water relations are largely governed by the plant internal water status depending on soil-plant and plant-atmosphere water flux. The plant internal water status is also a key factor in plant metabolism, and hence its growth and productivity (Zahran, 1989; Dosh, 1993 and Tramontaino and Jouve, 1997).

El-Shourbagy (1964) and Abd El-Fattah (1994). Found that there was reduction in the amino acids (number as well as concentration) in the plants growing under saline condition. Accumulation of some amino acids in response to drought and salinity has been observed in many plants (Ahmed *et al.*, 1981 ; Aspinali and Paleg, 1981).Moreover Laliberte and Hellebust (1989), found progressive accumulation of proline in response to salinity.

Morsy (1996). Found that the drought of soil resulted in marked increase in alanine, arginine, histidine, iso-leucine, valine, glutamic acid, phenylalanine and proline in the certain medicinal plants. Moreover,Singh *et al.*, (1973)found that proline was the most abundant amino acid accumulated in barley in response to water stress.

Belesky *et al.*, (1984) found that significant increases in proline, phenylalanine (cyclic amino acids), glutamatic, aspartic (acidic amino acids), therionine (amino acid contains S-group), alanine, serine and valine (aliphatic amino acids) concentrations in tall Fescue plant occurred under low soil water availability , compared with adequate water conditions.

Many plants and other organisms cope with osmotic stress by synthesizing and accumulating some compatible solutes, which are termed as osmoprotectants or osmolytes. These compounds are small, electrically neutral molecules, which are non-toxic even at molar concentrations (Alonso *et al.*, 2001). During osmotic stress, plant cells accumulate solutes to prevent water loss and to re-establish cell turgor. The solutes that accumulate during the osmotic adjustment include ions such as K^{+},Na^{+}, and Cl^{-} or organic solutes that include nitrogen containing compounds, such as proline and other amino acids, polyamines and quaternary ammonium compounds (Tamura *et al.*,2003 and Reddy *et al.*, 2004)

Drought tolerant plants can use several mechanisms to adapt water stress. These include reduction in water loss by increased stomatal resistance or increased water uptake by the development of large and deep root systems, increase in cyclic amino acid (Dubey, 1994 and Nour El-Din 1995).

The study of plant responses to water stress has been studied by physiologists who attempts to understand how plants function in their natural environment (Osmond *et al.,* 1987). Plants either avoid or tolerate periods of drought, often accompanied by high temperature and excessive irradiance levels (Ehleringer and Cooper, 1992), through phonological, morphological and physiological adjustments (Turner, 1986 and Morsy 2002).

Halophytes are plants, which are able to live under elevated salinities in their growth media, the salinity level in which they grow varies from slight, brackish medium to severe and may reach levels above seawater salinity (Gallagher, 1985 and Zahran, 1982).

The present study aimed to elucidate some physiological adaptive responses of species under the influence of different habitat conditions. For this reason, protein accumulation or concentration, protein amino acid and protein electrophoresis in the studied species were carried out to identify various protective adaptive responses of species growing in drought and saline stresses.

MATERIALS AND METHODS

The plant materials were collected from the various habitat of Wadi EL-Gafra in mid-July 2003. The habitat types were as follows:

I. Xeric habitat

Included two xeric plants represented by *Panicum turgidum*, Forssk and *Lasiurus hirsutus*, Forssk. These plants were collected from ups tream portion of Wadi El-Gafra, near the high way at 63km west of Suez, Egypt.

II. Saline habitats

Involved both of *Tamarix mannifera*, Ehrenb. and *Nitraria retusa*, Forssk. Collected from Midstream portion of Wadi EL-Gafra. Just N-NW EL-Asher Min Ramadan City, Egypt.

1. Chemical Analysis:

1.1. Total crude protein%:

Determination of total nitrogen and total protein as expressed by mg/g dry wt. were estimated using kjeldahl method (James, 1995).

1.2. Protein Amino Acids:

The hydrolyzed protein amino acids were determined according to the method described by Pellet and Young (1980).Defatted plant powder was dissolved in 6N HCl in a sealing tube. The mixture was hydrolyzed at 110°C for 24 hours; then the hydrolyzate was dried under vacuum at 70°C, and dissolved in sodium citrate buffer(pH2), and injected in the Amino Acid Analyzer.

1.3. Protein electrophoresis:

S.D.S polyacrylamide gel electrophoresis (S.D.S. PAGE) was performed for total proteins of the four different species according to the method of Laemmli (1970), as modified by Studier (1973).

RESULTS AND DISCUSSION

1.1.2. Crude Protein and Protein Amino Acids:

Protein synthesis is closely related to production of new tissue, which is the principle sink for nitrogenous compounds, and it is not surprising that when water stress inhibits growth, nitrogen metabolism is disturbed. An overall decrease in the levels of total protein content in plants growing under water stress conditions compared with plants growing under non-stressed environments was documented (Ramanjulu and Suduhakar, 1995).

The results in Table (1) show that the total crude protein and protein amino acids concentrations of different plant species in two different habitat conditions. The data indicate variation among the different plant species and locations in amino acid numbers and concentrations.

The studied xerophytes and halophytes show generally marked interspecific variations in their building units of proteins. Each species has its own collective assortment and concentration of amino acids that varied in some respect or with other species.

Table(1) indicates that total protein content in *Nitraria retusa*(halophytic plants) reached to the maximum values (113.1 mg/g dry wt.),while reached to its minimum values (57.5 mg/g dry wt.) in *Lasiurus hirsutus* (xerophytic plant).

According to Bewely and Larson (1980), and Bewley (1981), the production or availability of substances (e.g. sugar, anions, amino acids including proline) to maintain bound water could be important features of desiccation tolerance.Very mild to moderate water-stress reduces the level of protein synthesis in drought sensitive vegetative tissue and protein synthesis does not recover in cells subjected to severe water loss.

The *Tamarix mannifera* accumulated the highest concentration of cyclic amino acids (Proline, Phenylalanine and Tyrosine) followed by *Nitraria retusa* (17.68 and 16.97mg/g d.wt), respectively.The role of proline in plants exposed to salt stress has been investigated by many authors (Soltani & Bernard, 1977 and Raper & Kramer, 1983).

Table (1). Total crude protein and protein amino acid contents of some different plants species which were collected from up and mid stream portion of wadi EL-Gafra.

Measurements		*Panicum turgidum*	*Lasiurus hirsutus*	*Tamarix mannifera*	*Nitraria retusa*
Total crude protein mg/g d.wt		78.1	57.5	107.5	113.1
Amino acid (mg/g d.wt)					
Acidic amino acid	Aspartic	14.5	7.30	7.11	6.83
	Glutamic	3.11	3.73	4.75	6.46
Basic amino acid	Lysine	10.5	3.10	4.02	3.98
	Histadine	2.42	2.24	3.36	3.25
	Arginine	------	2.30	3.10	2.90
Cyclic amino acid	Proline	8.42	10.3	12.3	12.1
	Phenylalanine	2.24	2.50	3.62	3.38
	Tyrosine	2.10	1.12	1.73	1.49
Amino acid contain S – group	Methionine	0.76	0.68	0.69	0.75
Aliphatic amino acid	Glycine	2.91	3.22	4.60	4.31
	Alanine	3.42	2.52	3.84	3.81
	Valine	2.91	2.66	4.55	4.28
	Leucine	4.23	2.11	5.56	5.25
	Isoleucine	1.63	2.20	3.29	3.09
	Therionine	2.11	2.51	3.50	3.30
	Serine	2.22	2.85	3.94	3.69
	Amino butaric acid	4.02	4.81	6.10	6.01
Total number of amino acid		16	17	17	17
Total concentrations of amino acid		67.43	56.15	76.09	74.88

Proline increased in both the halophytes *Tamarix tetraghne* and the glycophyte *Pisum sativum*, when grown at various levels of Na Cl, and thus considered as evidence that it may act as a cytoplasmic osmoticum. Also, it is the most stable amino acid resisting oxidative acid hydrolysis to toxins, and is the least inhibitory of cell growth among all amino acids. The bound water would increase due to the highly hygroscopic nature of proline (Reddy *et al.*,2004)

Also proline is known to be involved in reducing the photodamage in the thylakoid membranes by scavenging and /or reducing the production of $^1O_2$. Proline accumulation in plants is caused, not only by the activation of proline biosynthesis, but also by the inactivation of proline degradation, thereby resulting in a decrease in the level of accumulated proline in rehydrated plants. Proline degradation to glutamic acid via pyrroline-5-carboxylate in higher plants is catalyzed by proline dehydrogenase.(Reddy *et al.*,2004) .It can also be inferred that proline acts as a free radical scavenger and may be more important in overcoming stress than in acting as a simple osmolyte (Bohnert and Jenson,1996 ;Alonso *et al.*,2001; Pinhero *et al.*, 2001 and Tamura et al.,2003).

Results from Table (1) revealed that the increased of both glycine, serin and glutamic amino acids in plants grown in saline habitats became more accumulated than xeric habitats, were recorded 6.46 mg/g dry wt. in

Nitraria retusa and 4.75 mg/g dry wt. in *Tamarix mannifera* ,while reached to 3.73 mg/g dry wt. in *Lasiurus hirsutus* and 3.11mg/g dry wt. in *Panicum turgiodum* . This results are in agreement with that stated by Lawlor and Cornic, 2002; they reported that the accumulation of other amino acids like glycine, serine, and glutamate are known to regulate and integrate the metabolism in stressed photosynthetic tissues.

The total concentration of amino acids ranged from(76.09 mg/g d.wt) in *Tamarix mannifera* to (56.15mg/g d.wt) in *Lasiurus hirsutus*.

Roy-Macauley *et al.,* (1992) reported that water – stressed plants show a high protease activity under stressful conditions appears to be a part of an adaptive potential, since it also led to the accumulation of free amino acids which contribute to osmotic adjustment and is related to drought tolerance (Rahmanjulu *et al.,* 1994 and Dubey 1994).

1-3. Protein fractination or electrophoresis:

Sodium dodecyl sulphate polyacrylamide gel-electrophoresis (SDS-PAGE) is widely used to fractionate the proteins according to their molecular weight (Bhattly, 1982; Fullington *et al.,*1983 and Sharcbeen *et al.,* 1991).

The data presented in table (2) illustrated the results of SDS-PAGE of four studied species. The obtained result revealed that the molecular weights ranged between (98 KDa to 6 KDa) and exhibited a maximum number of (41) bands, which were not necessarily present in all different studied species, however, there were specific (3) bands present and designated as common bands no (33, 37 and 40) of about molecular weight (21,17 and 9 KDa) for both xerophytes and halophytes.

The results also indicated that the bands no. (4,7 and 12) of the molecular weight (94,73 and 55 KDa) were present in xerophytes (*Panicum turgidum* and *Lasiurus hirsutus*). The results led to the assumption that these bands represented as specific band for each corresponding habitat. The results are in agreement with those obtained by Ericson and Alfinito (1984), they reported that some protein bands were enhanced under drought stress.

The evidence of drought – resistance of plants investigated the synthesis of high molecular weight proteins. In crease of soluble proteins (high M. wt.) increases the surface exposed to water binding, as bound water is correlated to drought resistance (Larosa *et al.,* 1989 and Moons *et al.,* 1995).

In this respect, Pareek *et al.,* (1995), found that specific stress proteins accumulate in response to not just one but a number of different biotic or a biotic stress conditions for instance, HSP 90 (a group of HSPs with molecular weights in the range of 80 to 90 KDa) accumulates in response to drought stress.

It was noticed from Table (2) that the presence of the bands no. (22, 27, 35, 38, 39 and 41) associated with halophytes plants (*Tamarix mannifera* and *Nitraria retusa*) and molecular weight ranged between (38 to 6 KDa).

Table (2) Protein patterns of the studied species of xeric and halophytic plants, from up and Mid steam protein of wadi EL-Gafra.

Mol. Wt Marker KDa	*Panicum turgidum*	*Lasiurus hirsutus*	*Tamarix mannifera*	*Nitraria retusa*
	M. wt. KDa	M. wt. KDa	M. wt. KDa	M. wt. KDa
				98
97				
			95	
	94	94		
				83
			78	
66	73	73		
				67
	63			
		59		
			56	
55	55	55		
				54
			53	
			51	
			48	
				46
			44	
				42
		40		
	39			
36			38	38
		37		
				35
	33		33	
31		32		
			29	29
			28	
	25	25		25
		24		
			23	
22		22		
	21	21	21	21
		20		20
			19	19
	18			
	17	17	17	17
14			14	14
			11	11
	9	9	9	9
			6	6
Total bands	11	14	19	18

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محتوى البروتين والاحماض الامينية لبعض نباتات المراعـــى المتـــأثرة بظـــروف الاجهاد المختلفة

ناهد محمد نور الدين
مركز بحوث الصحراء

استهدفت الدراسة:

- دراسة تاثير ظروف البيئة المتباينة على كلا من المحتوى الكلى للبروتين والاحماض الامينية التى تدخل فى تكوين البروتين وايضا تم دراسة وتحليل التفريد الكهربى للبروتين.
- وقد تم اختيار وادى الجفرة لتباين واختلاف العشائر النباتية النامية به وتم تحديد نباتين لبيئتين مختلفتين هما البيئة الجفافية والاخرى الملحية.
- وكان اختيار كلا من نبات الثمام *Panicum turgidum* ونبـــات *Lasiurus hirsutus* الحدة ليمثلا البيئة الجفافية حيث تم تجميعهم من اعلى الوادى بالقرب من الطريـــق الســـريع. بينما تم اختيار كلا من نبات *Tamarix mannifera* الطرفة ونبـــات *Nitraria retusa* الغردق ليمثلا البيئة الملحية من منتصف الوادى (شمال- شمال غرب مدينـــة العاشـــر مـــن رمضان).

وقد اظهرت النتائج:

١- ان هناك تباين واختلاف فى تركيز محتوى البروتين الكلى بالنسبة للنباتات الجفافيـــة كانـــت مراكمة البروتين اقل من النباتات التى تعيش بالبيئة الملحية.

٢- لوحظ ان عدد الاحماض الامينية المتكون منها البروتين كانت ثابتة فى ثلاثةانواع موضـــوع الدراسة ولكن كان هناك اختلاف فى تركيز ومكونات الاحماض الامينية الخاصة بكل نبـــات لكى يستطيع التأقلم مع الظروف البيئية النامى بها.

٣- اظهر التفريد الكهربى للبروتين تباين واضح بين الانواع النباتية حيث عملت النباتـــات التـــى تعيش فى بيئة ملحية على تراكم البروتين ذو الاوزان الجزيئية الصغيرة بينما راكمت النباتات الجفافية البروتين ذو الاوزان الجزيئية الكبيرة. كما لوحظ اشتراك الاربـــع انـــواع النباتيـــة موضوع الدراسة فى بعض الحزم متشابهة الاوزان الجزيئية.