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Seasonal Changes in Soluble Proteins of Some Native Desert Species

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Abstract: This research was carried out on eight wild species inhabiting two oases in the Western Egyptian Desert. Plants in both regions were categorized into: a- Halophytes, namely: *Salsola imbricata, Cressa cretica,* and *Suaeda monoica,* b- Xerophytes include: *Alhagi graecorum, Hyoscyamus muticus, Prosopis farcta*, and *Gossypium arboretum* and c- Succulent *Zygophyllum coccineum*. The plant samples were collected at different sites during winter and summer seasons. Laboratory analyses on plants included total and specific soluble proteins. The resultsobtained indicated that: locations or its interaction with seasonality dominantly affect soluble proteins.Gel electrophoresis showed that the low molecular weight proteins had the high percentage. Halophytic species especially *C. cretica,* and *S.imbricata* had a relatively high molecular weight protein in summer while xerophytic species such as *P. farcta* and a succulent *Z. coccineum* had a relatively high molecular weight protein during winter.

Keywords: Drought, Halophytes, Osmotic Adjustment, Xerophytes, Succulents

1. Introduction

Drought, heat and salinity stresses are often combined in nature. Need to learn about plant responses to extremes in temperature, soil aridity, and salinity in various elements of desert vegetation can be a basis to judge for success or failure of any prospective crop plants to be introduced to such habitats in amelioration projects hoped for. In addition, the scientific information expected from such investigation are highly valuable concerning mechanisms of physiological adjustments available to the variety of species composing the natural vegetation in such habitats. When plants experience environmental stresses, such as drought, salinity and temperature, they activate various metabolic and defense systems to survive [1].

The adaptability of plant species to high salt concentrations in the soil by lowering tissue osmotic water potential was accompanied by accumulation of such osmotic solutes as soluble carbohydrates, proteins and free amino acids [2]. Salinity promotes synthesis of Salt Shock Protein (SSP), causes either increase or decrease in the level of total and soluble protein, depending on the plant parts studied and leads to increased activity of many enzymes [3]. However, proteins produced under salt stress are not always associated with salt tolerance; consequently, using proteins as a salt tolerance indicator depends on the nature of the plant species or cultivar [4]. These observations suggest the possible involvement of these polypeptides for osmotic adjustment under salt stress [5]. Also, Gomathi and Vasantha [6] concluded that changes in RNA and DNA content under salinity stress might be responsible for specific expression of Salt Shock Proteins with MW of 15, 28 and 72 kDa in tolerant genotypes, while there were completely absent in susceptible.

The study hitherto presented is an attempt to recognize some identifiable means of adjustment in desert vegetation of inland desert oases. Therefore, it is of great significance to investigate the molecular mechanism of salinity, drought and heat tolerance in plants and improve stress tolerance of introduced crops. Accordingly, the aim of the work presented

here was to investigate the changes of water soluble proteins (specific and total) in some desert species. The species investigated include basically those of different ecological affiliations as well as different life forms, in order to have comparative indications in the means of adjustment.

Study area:-

Kharga Oasis is located in the Western Desert of Egypt, about 200 km west of the Nile River, between latitudes 24° 30° N - 26° 00° N; longitudes 30° 07/ E - 30° 47/ E and covering an area of 7200 km^2**.** The Dakhla Oasis is located at about 190 km west of Kharga (390 km west the NileValley), between latitudes 25° 26/ 30// and 25° 45/ 12// North and between longitudes 28° 42/ 00// and 29° 25/ 48// East, covering an area of 3400 km^2 (Figure 1). The New Valley Governorate which includes Kharga, Dakhla, and Paris Oases lies at a distance of 600 kms from Cairo City and 225 kms from Assiut City, with a vast area of about 458,000 square kilometers (i.e. 45.8% of the total area of Egypt and about 67% of the western desert area).

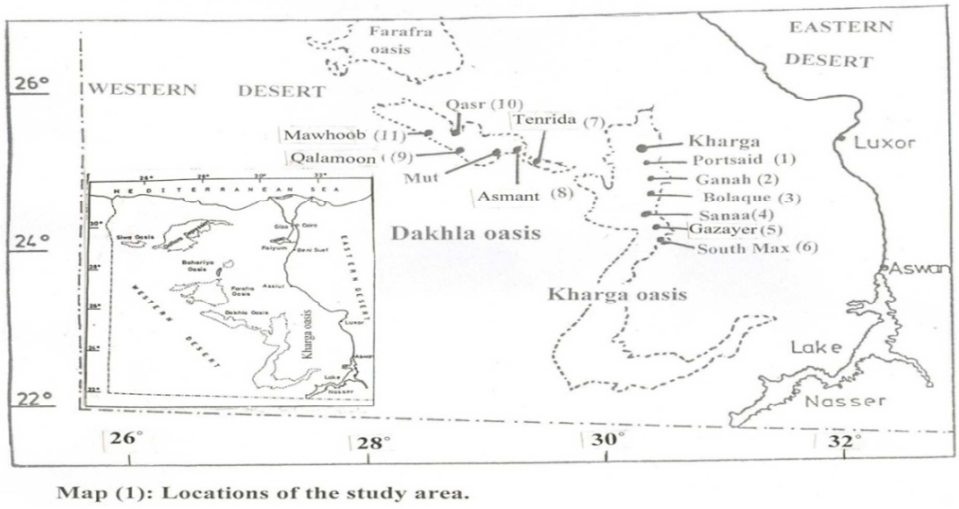


Figure 1. *Location of the study area.*

2. Materials and Methods

2.1. Collection of Plant Samples

Samples of 8 native species were collected from their natural habitats in the sites studied, when encountered. Plants were sampled twice: - 1- In January (representing mild winter conditions) and, 2- In September (representing the close up of harsh summer climate) in order to cover the seasonal changes in tested parameters in response to changes in climatic conditions. The studied species were identified according to Täckholm[7] and Boulos [8]. The investigated species including: *Alhagi graecorum* Boiss, (Family: Fabaceae), *Prosopis farcta (Bank & Sol.) Macbr* (F.: Mimosaceae), *Suaeda monoica* Forssk., *Salsola imbricate* Forssk., (F.: Chenopodiaceae), *Cressa cretica* L. (F.: Convolvulaceae), *Gossypium arboreum* L. (F.: Malaceae), *Zygophyllum coccineum* L. (F.: Zygophyllaceae) and *Hyoscyamus muticus* L. (F.: Solanaceae). The samples collected were branches bearing leaves, were immediately transferred to tightly close plastic containers, and transferred from their natural habitats to the laboratory. Samples of leaves were washed with distilled water and thoroughly dried on filter paper. For each species, four samples were chosen at random, then oven-dried at 70°C for 24 hrs.

2.2. Preparation of Plant Extracts for Analysis

One gram of finely powdered oven dried material of each plant sample was transferred to a clean test tube. Ten ml of bi-distilled water was added and heated to 80°C in a water bath for an hour, stirred at intervals and then filtration was done by using filter paper according to El-Sharkawi and Michel [9]. Plant extracts were kept in vials in deep freeze for chemical analyses.

2.3. Determination of Water Soluble Nitrogen Metabolites

Soluble proteins were determined according to Lowry *et al.,* [10]. Protein analysis by acrylamide gel electrophoresis (SDS-PAGE) was carried out according to Laemmli [11] in the first dimension, and the Lablmage program was used in determination of the molecular weight and percentage of water soluble proteins.

2.4. Statistical Evaluation of Experimental Data

The effects of single factors (season or location) and their interaction (season x location) on the contents of metabolites in different species were evaluated statistically by the analysis of variance (F test). The relative role of each single factor and their interaction in the total response were determined by using the coefficient of determination (η^2) to indicate the degree of control of the factor on the parameter tested [12]; [13] as applied by EL-Sharkawi and Springuel [14].

3. Results

3.1. Soluble Proteins (S.P.)

Soluble protein concentrations (mg/ml sap) exhibited marked differences in each species due to location and season (Figure, 2). Halophytes had higher soluble proteins than xerophytes and succulent species. Soluble proteins were higher in winter than in summer with some exceptions.

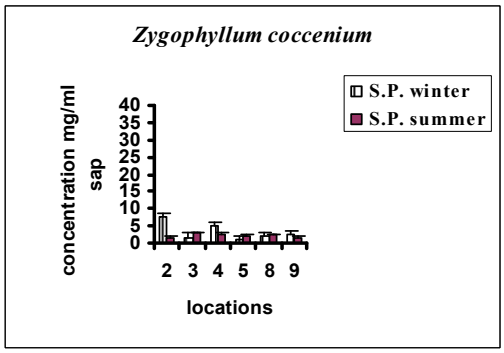
In summer, *Gossypium* (as xerophytic species) had the highest S.P. concentration followed by *Salsola* (as halophytic species), while *Hyoscyamus* (as xerophytic) had the lowest concentration. In winter, *Prosopis* (as xerophytic species) had the highest S.P. concentration. In *Zygophyllum* (a succulent species), a moderate concentration of S.P. ranging between were observed.

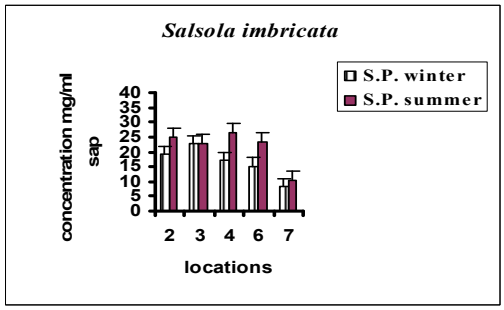
Data in table, 1 show a generally significant role of seasons, locations, and their interactions on soluble proteins concentration with some exceptions. Locations had a dominant effect on S.P. concentration of halophytic species, as well as in case of *G. arboreum* and *P. farcta*. Whereas, the (Se x Lo) interaction had a dominant role in case of *Zygophyllum*, *Hyoscyamus*, and *Alhagi*

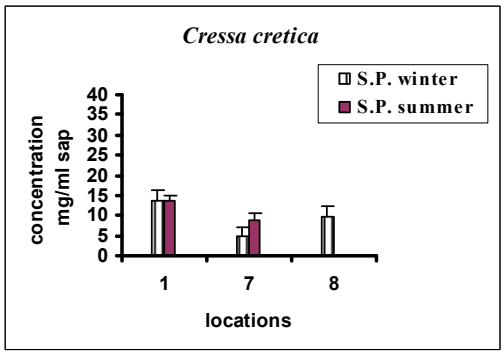
Table 1. *ANOVA test showed the effects of seasons, locations and their interaction on soluble proteins of investigated species at Kharga and Dakhla regions.*

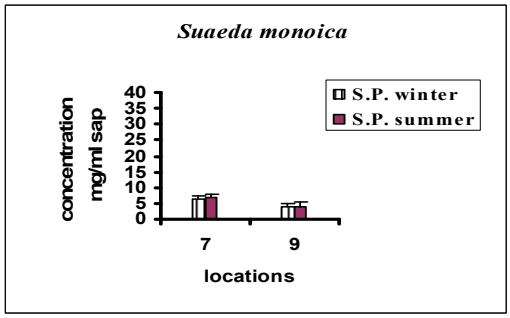
Contents / Species	Source of variance	Soluble proteins	
		F	η^2
	Seasons	10.01**	0.07
Salsola imbricata	Locations	26.1**	0.74
	Sex*Lo	6.73**	0.19
	Seasons	2.2	0.04
Gossypium arboreum	Locations	51.02**	0.88
	Se*xLo	4.76	0.08
	Seasons	8.27**	0.08
Zygophyllum coccineum	Locations	7.98**	0.41
	Se*xLo	10.11	0.51
	Seasons	0.55	0.03
Suaeda monoica	Locations	16.23**	0.97
	Se*xLo	0.006	0.00
	Seasons	0.36	0.02
Hyoscyamus muticus	Locations	0.16	0.03
	Se*xLo	4.82*	0.95
	Seasons	0.004	0.00
Alhagi graecorum	Locations	8.01**	0.48
	Se*xLo	8.59**	0.52
	Seasons	2.67	0.07
Cressa cretica	Locations	33.76**	0.84
	Se*xLo	3.79**	0.09
	Seasons	4.65*	0.07
Prosopis farcta	Locations	28.69**	0.87
	Se x Lo	1.89	0.06

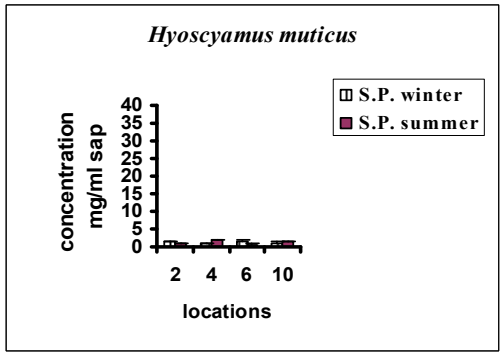
*Significant at P < 0.05 level. ** Significant at P < 0.01 level.











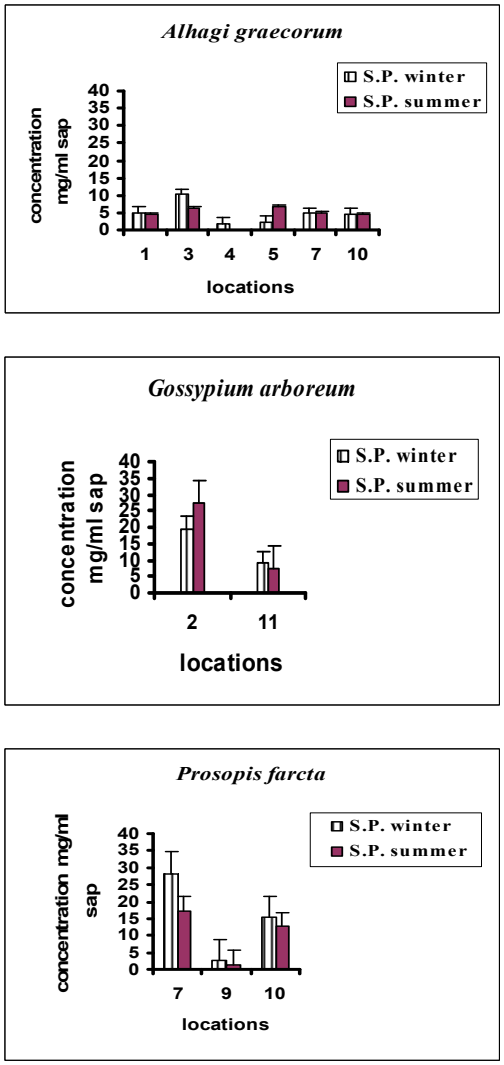


Figure 2. *The average content (mg.ml^{-1} sap) of soluble proteins (S.P.) in investigated species at different locations during winter and summer seasons.*

3.2. Specific Soluble Proteins (S.P.)

The SDS-PAGE of water soluble proteins fractions revealed outstanding differences in the banding profile patterns, represented by their presence or absence and intensity, in species chosen from specific representative habitats as shown in figures (3-6) of gel electrophoresis.

In *Z. coccineum*, 2-3 bands were detected in winter with molecular weights ranging from 23 to152 KD, low molecular weights 23 &44 KD with a percentage of 89.0% and 81.2% of the total, respectively. Four protein bands were found in summer having molecular weights ranging from 19 to 82 KD. It is striking that *Zygophyllum* showed high percentages (100%) of low molecular weight proteins (19 KD) in both seasons.

S. imbricate show 1-4 protein bands in winter having low and moderates molecular weights proteins (23 to 98 KD), low molecular weight is represented by high percentage. In summer, 2-4 protein bands were detected having different molecular weights (21 to 164 KD), new protein band of a high molecular weight (164 KD) appeared and a low molecular weight (21 KD) has a percentage of 75.1% of the total.

In *C. cretica*, 3 bands were detected with different molecular weights ranging from 25 to 89 K D (Table, 2) during winter and 53 KD has a percentage of 54.4%. In summer, 2-5 protein bands were detected with different molecular weights ranging between 21 and 190 KD and low molecular weights (21 KD) constitute 91.0% of the total (at site 1). Among these, two new protein bands were clearly observed in the hot season only which had a molecular weight of 149 KD representing 9% and 190 KD representing 48.8% of the total (at site 7).

In *S. monoica*, 3 protein bands were found in winter having low & moderate molecular weight (25-92 KD), low molecular weight has a percentage of 82.9%. In summer, protein bands with molecular weight ranging between 32 and 94 KD were detected. The data showed that the halophytic species *Salsola* and *Suaeda* maintained low molecular weight proteins in winter.

In *A. graecorum,* 3 bands were found during winter with molecular weights ranging from 24 to88 KD and the low molecular weight represents 63.7% of the total. In summer, protein bands increased to 5 bands with molecular weight ranging between 22 and 149 KD. This species may depend mainly on low molecular weight proteins where low M. wt. 22 KD represents 40.8% of the total.

H.muticus had 2-3 protein bands with molecular weights 23.48 KD in winter, and low M. wt. 23 &33 KD representing 69.8 % & 64.1% of the total, respectively. In summer, 2-4 protein bands were detected with molecular weights ranging between 21 and 142 KD. Likewise, in *Zygophyllum*, this species has a high proportion of low molecular weight proteins.

In *P. farcta*, 5 protein bands were found in winter having molecular weights ranging between 24 and169 KD, markedly high molecular weight proteins (169KD) are according to its percentage (Table 2). In summer, these bands were reduced to two bands containing low molecular weight proteins..

In *G. arboreum*, 3 protein bands were detected during winter and having molecular weights in the range 25-93 KD and low M. wt. 43 KD has a percentage of 85.1%.

4. Discussion

Plant adaptation to stress under natural conditions has some ecological advantages, the metabolic and energy costs may sometimes mask and limit its benefit to agriculture and result in yield penalty. Therefore, the improvement of abiotic stress tolerance of agricultural plants can only be achieved, practically, by combining traditional and molecular breeding [15]. Gel electrophoresis patterns of water soluble proteins suggested that most investigated species contain low molecular weight proteins and showed their formation with high percentage. Halophytes such as *Cressa* and *Salsola* formed relatively high molecular weight proteins in the range during summer while xerophytic species such as *Prosopis* and succulent plants such as *Zygophyllum* formed relatively

high molecular weight proteins during winter. *Hyoscyamus* and *Alhagi* have an exception that they can form relatively high molecular weight proteins in summer.

The response of plants to salt and other environmental stresses have been extensively investigated by proteomic approach for many decades, still we have not been able to understand fully the mechanism which imparts tolerance to some plants and sensitivity to others due to the complexity of the mechanism [15]. *Suaeda* (as halophytic species) form low and moderate molecular weight proteins and thus may play a role in water binding. In some cases, during summer, protein bands disappeared which may be due to the conversion of soluble proteins to free amino acids or it is degraded [16]; [17]; [18]. In *Cressa*, two new protein markers for drought tolerance were induced under high temperature (hot summer season). These results agree with finding by Abdel-Hady and El-Nagar [19] in wheat. Although Pareek *et al*., [4] suggested

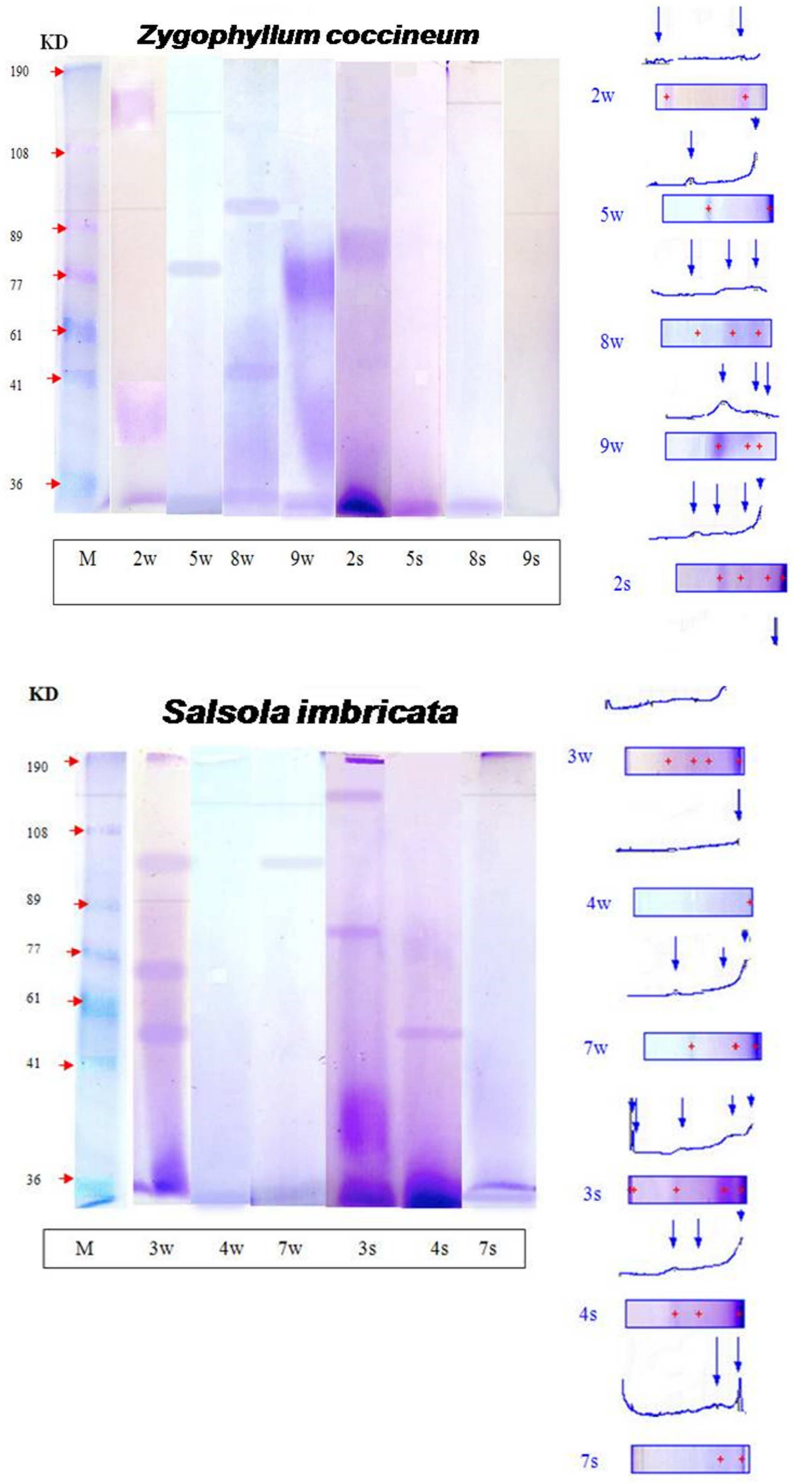


Figure 3. *Soluble protein patterns (Coomassie blue stain 12% SDS-PAGE gel) in Zygophyllum and Salsola during winter (W) and summer (S) at different sites.*

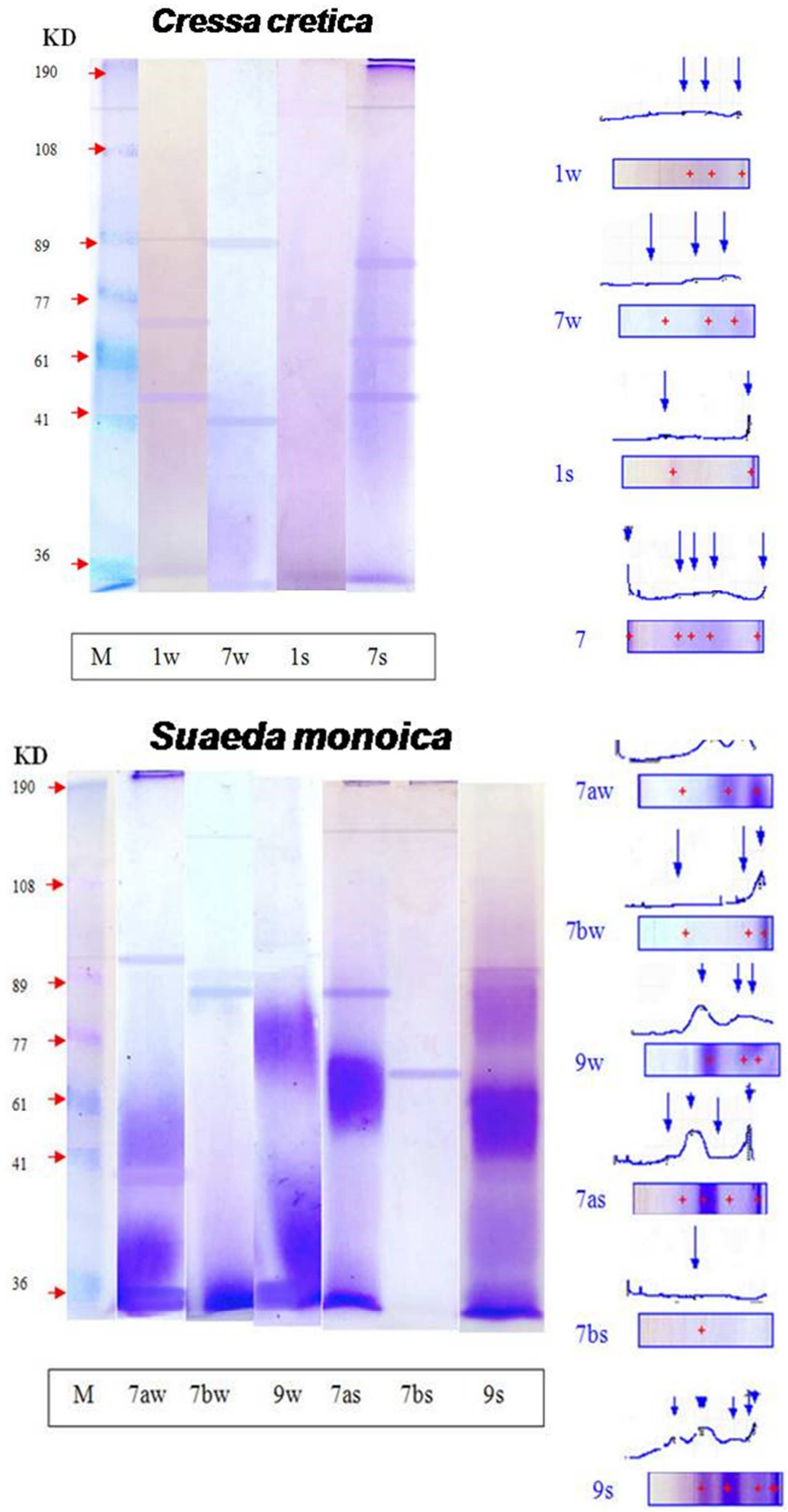


Figure 4. *Soluble protein patterns (Coomassie blue stain 12% SDS-PAGE gel) in Cressa and Suaeda during winter (W) and summer (S) at different sites.*

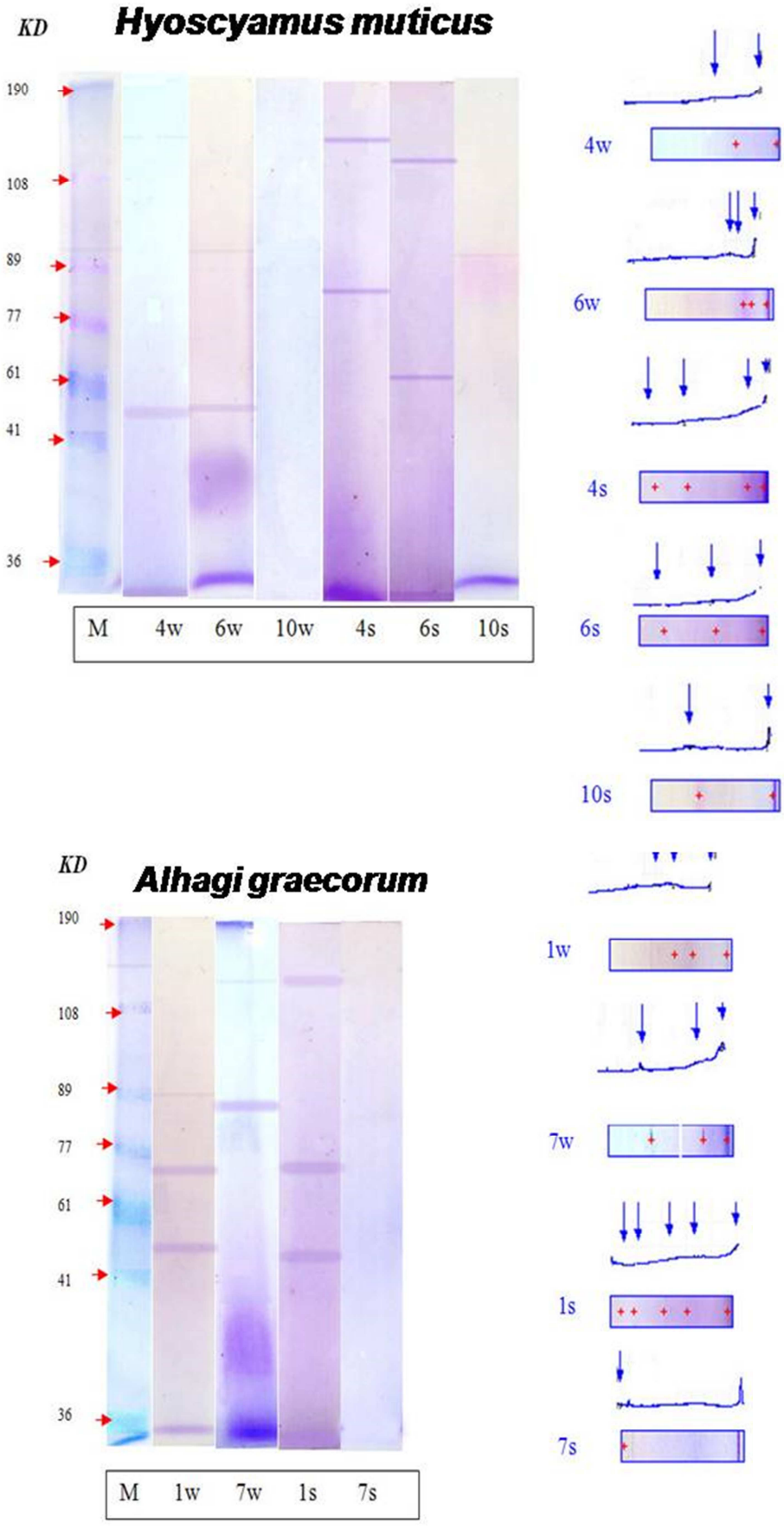


Figure 5. *Soluble protein patterns (Coomassie blue stain 12% SDS-PAGE gel) in Alhagi and Hyoscyamus during winter (W) and summer (S) at different sites.*

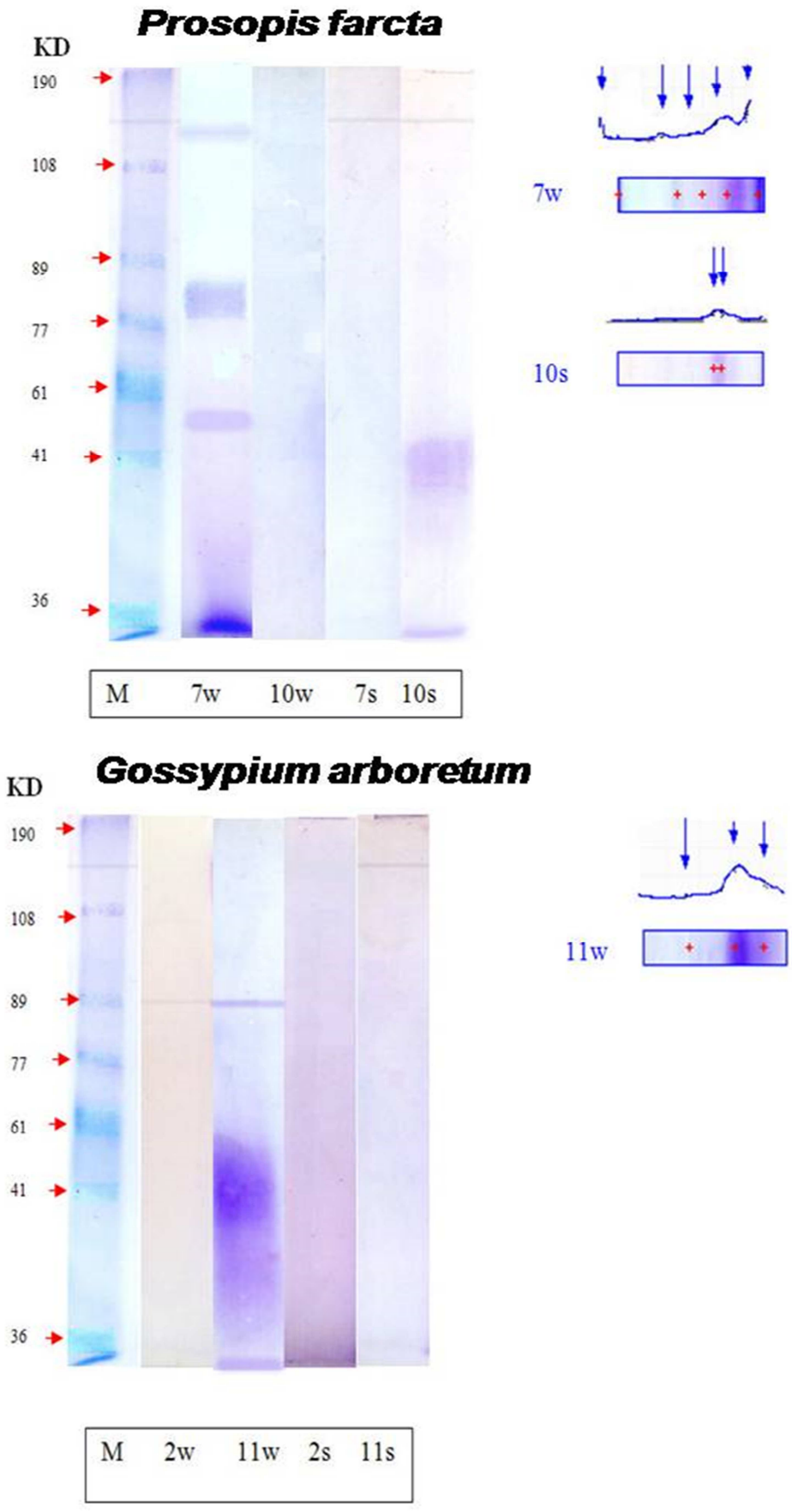


Figure 6. *Soluble protein patterns (Coomassie blue stain 12% SDS-PAGE gel) in Prosopis and Gossypium during winter (W) and summer (S) at different sites.*

Table 2. *Gel electrophoresis showing molecular weight of proteins in different species at different locations and their percentage during the two seasons.*

Seasons / Species	Locations	Winter			Summer			Winter	Summer
		Band number	Percentage (%)	Molecular weight (KD)	Band number	Percentage (%)	Molecular weight (KD)	No. of band	No. of band
Cressa cretica	1	1	22.434	70	1	8.984	149	3	2
		2	54.401	53	2	91.016	21		
		3	23.166	33					
	7	1	15.149	89	1	48.384	190	3	5
		2	32.625	41	2	10.225	83		
		3	52.227	25	3	12.183	67		
					4	13.824	49		
					5	15.385	21		
Alhagi graecorum	1	1	21.819	74	1	10.944	149	3	4
		2	52.825	57	2	23.469	75		
		3	25.356	32	3	24.742	49		
					4	40.846	22		
	7	1	13.682	88	-	-	-	3	-
		2	22.611	38					
		3	63.706	24					
Gossypium arboreum	2	-	-	-	-	-	-	-	-
	11	1	3.948	93	-	-	-	3	-
		2	85.104	43					
		3	10.947	25					
Zygophyllum coccineum	2	1	18.812	152	1	7.204	82	2	4
		2	81.188	44	2	6.630	54		
					3	9.323	30		
					4	76.843	21		
	5	1	10.967	78	1	100	21	2	1
		2	89.033	23					
	8	1	13.872	92	1	100	19	3	1
		2	40.645	42					
		3	45.484	36					
	9	1	61.164	65	1	100	32	3	1
		2	22.167	33					
		3	16.669	25					
Hyoscyamus muticus	4	1	30.225	47	1	6.713	142	2	4
					2	10.097	85		
		2	69.775	23	3	21.176	30		
					4	62.014	21		
	6	1	21.383	48	1	19.294	121	3	3
		2	14.497	42	2	27.107	61		
		3	64.120	33	3	53.599	21		
Prosopis farcta	10	-	-	-	1	27.084	98	-	2
					2	72.915	33		
	7	1	39.725	169	-	-	-	5	-
		2	6.970	89					
		3	5.062	57					
		4	19.607	39					
		5	28.635	24					
	10	-	-	-	1	44.814	41	-	2
					2	55.186	38		

Table 2. *Continue: Gel electrophoresis showing molecular weight of proteins in different species at different locations and their percentage during the two seasons.*

Seasons / Species	Locations	Winter			Summer			Winter	Summer
		Band number	Percentage (%)	Molecular weight (KD)	Band number	Percentage (%)	Molecular weight (KD)	No. of band	No. of band
	3	1	16.955	98	1	2.357	164	4	4
		2	21.189	70	2	10.803	79		
		3	12.180	54	3	20.323	32		
		4	49.677	33	4	66.517	21		
Salsola imbricata	4	1	100	23	1	11.607	78	1	3
					2	13.283	51		
					3	75.110	21		
	7	1	9.780	89	1	34.329	34	3	2
		2	30.175	37	2	65.671	36		
		3	60.044	36					
	7a	1	2.500	92	1	12.625	83	3	4
		2	14.609	40	2	33.427	58		
		3	82.891	36	3	11.692	37		
					4	42.256	36		
Suaeda monoica	7b	1	9.000	88	1	100	67	3	1
		2	19.095	33					
		3	71.905	25					
	9	1	32.716	65	1	12.135	94	3	5
		2	46.460	35	2	32.843	68		
					3	23.477	44		
		3	20.824	25	4	13.086	35		
					5	18.459	32		

Location numbers: 1-Portsaid, 2- Ganah, 3- Bolaque, 4-Sanaa, 5- Gazayer, 6-South Max, 7 a&b -Teneida, 8- Asmant, 9-Qalamoon, 10- Qassr, 11- Mawhoob at Kharga and Dakhla regions.

that, stress proteins could be used as important molecular markers for improvement of salt tolerance using genetic engineering techniques; in many studies the proteins produced under salt stress are not always associated with salt tolerance.

Thus using proteins as salt tolerance indicators depends on the nature of the plant species or cultivar [20]. Also, thermo-tolerance against heat stress has been accomplished in plants transferred with heat shock regulatory proteins [21]. Moreover, the adaptation of these plants to heat stress induced accumulation of water binding molecules and compatible solutes which related to enhance thermostability [22]. Commonly, the significant effect of season, location, and their interaction on soluble proteins concentration was detected in most investigated species with some exceptions. Accordingly, a larger content of soluble proteins (S.P.), resulting in binding water molecules is found in *S. imbricate* and *G. arboreum* particularly in summer. Meanwhile, *P.farcta* accumulate S.P. in winter. This accumulation is beneficial in maintaining the viscous properties and contributes to increasing osmolality of the cytoplasm [23], [24].

5. Conclusion

Finally, locality or its interaction with seasonality had a predominant role on the total soluble proteins of species exposed to drastic condition. Furthermore, the gel electrophoresis showed that the low molecular weight proteins had the high percentage among the investigated species in order to enhance thermostability. The halophytic species had a relatively high molecular weight protein in summer, while xerophytic and a succulent species had relatively high molecular weight proteins during winter.

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