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Seasonal Variations in Protein Patterns and Mineral Contents of *Lycium showii* under Different Habitat Conditions

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Two locations Wadi El-Bagha in South Sinai and Wadi Hashem in Mersa Matruh, Egypt were selected for monitoring changes of protein patterns and chemical composition of *Lycium showii* (*L. showii*) due to seasonal variations. Significant differences ($P < 0.05$ or 0.01) occurred of mechanical properties and chemical analysis of the soil associated of *L. showii* by 0-20 and 20-40 cm depths from three sites (up, mid, down-streams) in Wadi El-Bagha and Wadi Hashem as well as interactions them (depths, sites and locations). Soil associated with the plants in Wadi Hashem possessed higher water content and electrical conductivity in down-stream as well as higher Cl^-, Ca^{2+}, Mg^{2+}, Na^+ and K^+ in the up-stream during the 20-40 cm depth. Locations, sites and seasons as well as their interactions trends were showed highly significant with plant Na^+, K^+, Ca^{2+}, Mg^{2+}, N and P contents, with insignificance in Ca^{2+} and N contents by seasons and locations x seasons, respectively. The Na^+ and K^+ of Wadi Hashem and N and P of Wadi El-Bagha in autumn as well as Ca^{2+} of Wadi Hashem and Mg^{2+} of Wadi El-Bagha in spring were recorded the highest values during mid-stream. The SDS-PAGE method showed different molecular weights of protein patterns in *L. shawii* leaves in the two locations during autumn and spring seasons. The highest molecular

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weight (148.3 kD) was observed in Wadi El-Bagha during autumn season, while the lowest molecular weight (10.5 kD) was found in Wadi Hashem and Wadi El-Bagha during spring and autumn season, respectively. The number of bands in Wadi El-Bagha had higher than in Wadi Hashem during the both seasons. The leaves of *L. showii* have specific unique high molecular weights proteins in Wadi El-Bagha at autumn and spring seasons. Thus, these patterns reflect variations of behavior and adaptation of *L. shawii* under stress conditions in the studied locations and seasons.

Keywords: Protein patterns; SDS PAGE; Chemical composition; Lycium shawii.

1. INTRODUCTION

The degradation of soils, drought, global climatic warming and the loss of perennial palatable species, overgrazing and human induced activities lowers the productivity of ecosystems and reduces the species richness and relative abundance [1,2]. Environmental conditions affect not only plant growth but also influences secondary metabolites. The medicinal plants show a marked variation in active ingredients during different seasons; as these have been widely attributed to variations in environmental variables such as temperature and rainfall [3].

Solanaceae is large and economically important family of herbs or shrubs or trees often strongly scented and sometimes narcotic or poisonous. In Egypt Solanaceae is a well-represented family, about 30-33 wild species belonging to eight genera according to [4]. Moreover Hepper [5] reported that the family is represented by 25 genera and about 91 wild and cultivated species. *Lyceum Shawii* Roem & Schult (*L. Shawii)* belong to Solanaceae and it is used as source of food and traditional medicine. Decoction of boiled roots is exercised in coughs and for sores treatments, as well as, to wash polio patients in case of a cut. Its leaves are used in treatment of constipation and stomach ache. Moreover, the branches are utilized for fencing as well [6].

To cope with the effects of salt stress, plants have evolved many biochemical and molecular mechanisms to reduce detrimental effects of ions from those parts of the plants where they may be harmful; these mechanisms include accumulation at the root level, the shedding of dry leaves, salt secretion and succulence [7]. Osmotic adjustment in plants can be performed through accu-mulation of osmolytes which are compatible with the cells metabolism [8]. Low water availability in soils may have different effects on soil nutrient concentration, although nutrient concentration may increase when the volume of soil water declines and leads to improved uptake by plants for a short period [9]. All soils contain a mixture of soluble salts; the most common cations associated with soil salinity are Ca^{2+}, Mg^{2+} and Na^{+} [10].

In addition to the metabolized mineral elements N, P and S, plants require Ca^{2+}, Mg^{2+} and K^{+} in relatively large amounts (>0.1% of dry mass), and each of these so-called macroelements is an essential plant nutrient [11]. The chemical composition of pasture components can follow a seasonal pattern which is more evident in some constituents than in others. It is clear that the major variations among chemical compositions observed represent genuine seasonal trends superimposed on random fertility, maturity, or species effects, also the magnitude of effects evidently differs a good deal among the various chemical parameters [12,13].

Molecular biology has revolutionized the field of plant systematics and has been applied successfully in phylogenetic relationships at all taxonomic levels [14], in genetic diversity studies [15], to studying the genetic relationships among plants [16] and to determine stress-induced proteins in plant species [17]. One way to understand the ability of plants to tolerate environmental stresses (drought, salinity and different temperatures) is to study and identify the changes at specific protein levels caused by stress [18]. Method of Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis analysis (SDS-PAGE) is a standard method applied to separate, identify and purify nucleic acids, since this gel is porous in nature. Using SDS-PAGE method, Arora et al. [19] reported that drought stress induced the accumulation of heat stress responsive proteins belonging to dehydrin group (25-60 kD) and/or aquaporins (25-30 kD). Protein pattern gel electrophoresis study revealed the appearance of most proteins that have specific low molecular weights, which are unique for each plant. While, there are some plants have specific unique high molecular weights proteins during dry season. These patterns reflect variations of behavior and adaptation of these species under drought or salt stress conditions [20,21]. The main objective of this study was to investigate the effects of seasonal variations on protein patterns and chemical composition of *L. showii* under different habitat conditions.

2. MATERIALS AND METHODS

2.1 Study Area and Plant Material

This study was carried out in the two locations Wadi El-Bagha (29° 28' 23" N, 33° 6' 2" E) and Wadi Hashem (31° 21' 775" N, 27° 00' 476" E) in South Sinai and Mersa Matruh, Egypt, respectively. *L. Shawii* Roem & Schult is a flowering plant belonging to the genus Lycium, tribe Lycieae, subfamily Solanoideae and family Solanaceae according to the classification of Hunziker [22]. It is deciduous and evergreen shrubs often spiny; cosmopolitan in temperate and subtropical regions. It is grows up to 3m height. It is leaves do narrow towards its base. It produces small whitish pink or purple flowers and red pea sized seedy barriers that are edible [6].

2.2 Soil Analysis

Samples collection of soil associated with *L. Shawii* were carefully made from three random points at two depths (0-20 cm and 20-40 cm) in three sites (up, mid and down streams) from two different locations. Three replicates were taken from each sample and carried to the laboratory in closed tins to been used for soil analyses. Soil samples were air dried, sieved and used for mechanical analysis of soil particles as suggested by Jackson [23] and Rowell [24] for soil texture. The soil moisture content was calculated according to the method described by Rowell [24]. Electrical conductivity (EC) and pH value for each sample were carried out using soil-water paste, according to Jackson [25], EC was expressed as mmhos/cm. The mineral contents of soil including Cl^-, Ca^{2+}, Mg^{2+}, Na^+ and K^+ were estimated using a saturation paste [26].

2.3 Plant Analysis

2.3.1 Mineral contents

Three samples of *L. Shawii* were collected from three sites (up, mid and down streams) of two studied locations during the spring (March) and autumn (September) seasons of 2019. Drying of collected plant materials were done in the oven at 70°C to a constant weight after which dried samples were milled to fine powder and stored in brown bags at room temperature pending chemical analyses. The concentrations of Na^+ and K^+ were determined using a flame photometer, Ca^{2+} and Mg^{2+} were determined by versinate titration method as described by Rowell [24]. Shoot nitrogen was determined using the micro-Kjeldahl method [27], and phosphorus contents were estimated spectrophotometerically following the methods of Chapman and Pratt [28].

2.3.2 Protein gel electrophoretic

The protein from four samples of *L. Shawii* leaves during both studied seasons and locations were isolated using a modified sequential extraction standard procedure developed by Curioni et al. [29].

- ***SDS-PAGE Procedure***: Each sample was subjected to Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis analysis (SDS-PAGE) by following the basic method developed by Laemmli [30] and modified by Singh and Shepherd [31]. The dried protein pellets were solubilized in 250 µL of a sample buffer. The electrophoresis was carried out using a 10% gel concentration [30]. A 10-well, 0.75 mm comb was used in a Bio-Rad Mini Protein 3 System having gel size 8.3–7.3 cm. The SDS-gels contained 4% polyacrylamide stacking gel and a resolving gel of 10% polyacrylamide. Samples (30 µL aliquots from sample [5 mg] extracted with 250 µl of sample buffer) were applied into precast application slots.
- ***Detection of protein Bands and Gel Imaging***: Upon the completion of electrophoresis, the proteins were fixed in methanol/acetic acid/water (40/10/50). Then staining with Coomassie Blue R-250. 200 ml of the destaining solution was used to destain the gel. The gel was gently agitated on a shaker for 2 hours. This destaining procedure was repeated several times until the background color of tile gel was removed. Total bands for each species were scored and their molecular weight (Mol. Wt.) calculated using the protein marker as standard. The gel scanning was done on Helena Jonior 24 photo scanner and the data were integrated using the scanner software. On the first well of each gel,

the proteins were employed as the molecular weight (Daltons) markers ranging from 10–250 KDa.

2.4 Statistical Analysis

The data collected were evaluated using analysis of variance and the significant differences were identified using the Fisher's least significance difference (LSD) of ANOVA at significant difference at $P \leq 0.05$ and $P \leq 0.01$ [32]. Cluster analysis was done using a computer software program PAST version 2.17c. Statistical analysis was performed using SPSS 20.0 program.

3. RESULTS AND DISCUSSIONS

3.1 Soil Analysis

Analyses of variance (ANOVA) for mechanical properties of the soil associated with *L. showii* from the two depths in three different sites (up, mid and down streams) and their averaged across Wadi El-Bagha and Wadi Hashem locations are presented in Table 1. Most mechanical properties (%) were significantly ($p<0.05$ or $p<0.01$) affected by locations, sites, depths, the first order interactions (locations x sites, locations x depths and sites x depths) and the second order interaction (locations x sites x depths). This conformed to the earlier findings of El-Absy et al. [33] in *Achillea fragrantissima* and *Artemisia judaica* and El-Absy and Kamel [34] in *Teucrium polium* under different habitat conditions. The soil associated of *L. showii* in Wadi El-Bagha contained a high percentage of coarse sand followed by fine sand and medium sand at the two studies depths in the three studied sites (up, mid and down streams). While, the soil of Wadi Hashem was recorded the highest percentage of medium sand followed by fine sand and coarse sand at the depths in the sites. Therefore, the soil associated of *L. showii* and collected from the two depths and three sites in the two studied locations are sandy in texture. Bonifacio and Morte [35] reported that in arid environments, poor fertility conditions being linked to the sandy texture with low inputs of organic matter.

The water content showed highly significant differences between locations, sites and depths during spring and autumn seasons. While, the locations x sites interaction in autumn as well as the locations x depths and sites x depths interactions in spring were exhibited significant differences ($P < 0.01$) for water content under studied habitats (Table 1). Slight variation of saturation point was found between seasons at different depths by Abdel Kawy [21]. The values of water content from depths and sites in Wadi Hashem were higher than in Wadi El-Bagha, as well as the water content in 20-40 depth was greater than in 0-20 depth at studied habitats during spring and autumn seasons. Highest water content was recorded at 20-40 cm from mid-stream in Wadi El-Bagha and from down-stream in Wadi Hashem during spring and autumn seasons. Changes in soil water content could become both beneficial (increased P availability) to plant nutrition [9]. Higher variations at the surface depth are to be expected because this part of the soil profile is influenced by soil evaporation, which peaks in day time and is low at night time [36]. Generally, the values of water content in spring season had higher than in autumn season under studied habitat conditions. The results agreed with the view of *L. shawii* by Ahmed et al. [37], that the water content reached maximum values in winter and minimum values in summer due to rainfall, which lead to normal plant growth. This may be due to increased accumulation of total ion as a result of increased soil salinity and soil moisture stress [38].

The means and ANOVA for chemical analysis of the soil associated of *L. showii* under different habitats are given in Table 2. The factorial ANOVA revealed significant differences ($P < 0.05$ or 0.01) among the three factors (locations, seasons and sites) and their interactions (first and second order interactions) for chemical analysis i.e., electrical conductivity (EC), Cl^-, Ca^{2+}, Mg^{2+}, Na^+ and K^+, except sites x depths interaction for Mg^{2+} and Na^+ elements as well as second order interaction (locations x seasons x sites) for Ca^{2+}, Mg^{2+} and Na^+ elements which were non-significantly different. Similar findings were reported by El-Absy et al. [33] and El-Absy and Kamel [34]. The ANOVA indicates only one highly significant effect between locations on pH, while other factors and interactions were non-significant in their effects on pH. These results are in agreement with El-Lamey [39] who reported no significant differences in soil pH due to changes of location. All studied chemical analysis of the soil associated of *L. showii* in Wadi Hashem were higher than in Wadi El-Bagha during the two depths and the three sites, except pH which showed the opposite, which means that Wadi Hashem representing a saline one. The highest values of pH, EC and Na^+ in the first depth from down-stream, Ca^{2+} and Cl^- in the first depth from up-stream, and Mg^{2+} and K^+ in the first and second depths from mid-stream, respectively were obtained in Wadi El-Bagha. While, the highest values in Wadi Hashem were observed for pH in the first depth from mid-stream, EC in in the second depth from down-stream and other studied elements in the second depth from up-stream.

Table 1. Mechanical properties of the soil associated of *L. showii* at the two depths from three sites in Wadi El-Bagha and Wadi Hashem

Locations (L)	Sites (S)	Depths cm (D)	Mechanical Properties (%)								Water Content	
			Very Coarse Sand	Coarse Sand	Medium Sand	Fine Sand	Very Fine Sand	Slit	Clay	Soil Texture Class	Spring	Autumn
Wadi El-Bagha	Up	0-20	37.27	25.35	17.32	10.20	7.66	1.08	1.12	Sandy	3.64	2.55
		20-40	32.98	30.01	15.67	13.48	6.82	0.92	0.12	Sandy	4.64	3.72
	Mid	0-20	34.84	27.24	18.32	9.43	7.74	1.31	1.12	Sandy	6.65	4.93
		20-40	29.93	33.98	15.65	10.47	6.81	2.13	1.03	Sandy	8.82	6.65
	Down	0-20	26.95	31.32	13.13	9.46	5.71	7.12	6.31	Sandy	5.73	3.64
		20-40	28.36	26.75	11.83	13.22	5.58	9.12	5.14	Sandy	6.63	4.64
Wadi Hashem	Up	0-20	0.28	12.51	45.13	28.37	0.71	10.8	2.20	Sandy	7.39	5.28
		20-40	0.36	15.66	49.80	23.89	0.78	7.71	1.80	Sandy	7.86	7.39
	Mid	0-20	0.31	10.77	50.08	30.04	0.80	6.90	1.10	Sandy	7.71	6.46
		20-40	0.35	12.37	54.40	27.29	0.84	3.91	0.84	Sandy	9.69	7.92
	Down	0-20	0.29	15.01	50.05	24.40	0.74	5.41	4.10	Sandy	9.76	8.37
		20-40	0.39	12.51	53.04	28.41	0.75	2.11	2.79	Sandy	10.34	9.26
L.S.D. at 0.05 (*) and 0.01 (**)		L	**	**	**	**	**	**	*		**	**
		S	**	*	**	*	**	**	**		**	**
		D	**	**	**	**	*	**	**		**	**
		L x S	**	**	**	**	**	**	**		NS	**
		L x D	**	**	**	**	*	**	*		**	NS
		S x D	**	**	NS	**	NS	*	*		**	NS
		LxSxD	**	**	**	**	NS	**	NS		NS	NS

Table 2. Chemical analysis of the soil associated of *L. showii* at the two depths from three sites in Wadi El-Bagha and Wadi Hashem

Locations (L)	Sites (S)	Depth cm (D)	Chemical Analysis						
			pH	EC (dS/m)	Cl^-(meq/L)	Ca^{2+} (meq/L)	Mg^{2+} (meq/L)	Na^+ (meq/L)	K^+ (meq/L)
Wadi El-Bagha	Up	0-20	7.73	2.16	3.32	2.41	1.11	1.24	0.52
		20-40	7.75	0.63	3.21	1.46	1.01	1.15	0.55
	Mid	0-20	7.81	2.23	3.19	2.30	1.15	2.79	0.58
		20-40	7.69	0.71	3.16	1.41	1.06	2.73	0.61
	Down	0-20	7.98	4.56	1. 41	1.83	0.71	3.81	0.47
		20-40	7.80	2.72	1.02	1.64	0.82	3.73	0.49
Wadi Hashem	Up	0-20	7.42	6.87	7.39	8.52	19.64	14.75	7.19
		20-40	7.17	5.83	16.19	17.35	23.76	20.53	30.74
	Mid	0-20	7.75	4.86	6.61	7.61	16.62	10.68	4.73
		20-40	7.59	5.85	13.58	15.48	19.35	17.49	21.43
	Down	0-20	7.32	6.64	7.71	6.19	17.48	12.57	5.37
		20-40	7.14	7.52	15.48	13.74	20.85	19.19	18.49
L.S.D. at 0.05 (*) and 0.01 (**)		L	**	**	**	**	**	**	**
		S	NS	*	**	**	**	**	**
		D	NS	**	**	**	**	**	**
		L x S	NS	**	**	**	**	**	**
		L x D	NS	**	**	**	**	**	**
		S x D	NS	**	*	*	NS	NS	**
		LxSxD	NS	**	*	NS	NS	NS	**

Abdel Kawy [21] mentioned that EC was more than 2 mmhos in the soil associating with *L. europaeum*, indicating slight saline one, who add, the most minerals in soil was present at the surface layer only during dry season. In soils of neutral pH, the concentrations of Ca^{2+}, Mg^{2+} and nitrate are fairly high, whereas those of ammonium and particularly phosphate are very low [40]. The adjustment of the nutrient solution in terms of EC is crucial for the optimization of the water and nutrient availability [41]. High concentrations of Na^+ and Cl^- in the soil solution may depress nutrient-ion activities and produce extreme ratios of Na^+/Ca^{2+}, Na^+/K^+, Ca^{2+}/Mg^{2+} and Cl^-/NO_3^- [42]. The excessive accumulation of salts may cause a change on the uptake of mineral nutrients as well as induce phytotoxicity [43].

3.2 Plant Analysis

3.2.1 Mineral contents

The sites and seasons were two distinct factors to access the significant differences among sites and seasons under Wadi El-Bagha and Wadi Hashem for chemical compositions concentrations (%) of *L. showii* (Table 3). Differences due to locations, seasons, sites, the first order interactions (locations x seasons, locations x sites and seasons x sites) and the second order interaction (locations x seasons x sites) were highly significant ($p<0.01$) for Na^+, K^+, Ca^{2+}, Mg^{2+}, N and P concentrations except seasons for Ca^{2+} and locations x seasons for N were exhibited insignificance. These results corroborates the results obtained by Abdel Kawy [21], El-Absy and Kamel [34], Estevez et al. [44] and El-Lamey [39] who mentioned that the minerals concentrations were exhibited significant differences among studied habitats, especially under stress conditions. Addition, obvious seasonal trends occurred with plant mineral composition N, P, Mg^{2+}, K^+ and Ca^{2+} [12] as well as with N, P and Ca^{2+} [45], with not found seasonal trends in Na^+ [12] and with less well defined seasonal variations in K and Mg contents [45]. This may reflect seasonal changes in physiological needs and effort, rather than availability in plant content [44].

The values of Mg^{2+}, N and P concentrations in Wadi El-Bagha were higher than in Wadi Hashem during the three sites (up, mid, down-streams). While, the values of Na^+, K^+ and Ca^{2+} concentrations in Wadi Hashem were higher than in Wadi El-Bagha during up and mid-streams (Table 3). Most studied elements were greater in autumn than in spring during the two studied locations. The maximum Na^+ and K^+ minerals were recorded in the autumn season and Ca^{2+} mineral in the spring season during Wadi Hashem. On the other hand, Mg^{2+} and N elements in the autumn season and P element in the spring season during Wadi El-Bagha showed the highest values. The least values were found for Na^+, Ca^{2+}, Mg^{2+} and P minerals in spring season, K^+ and N minerals in autumn seasons during Wadi El-Bagha. The highest amount of total nitrogen content was recorded in winter which may be due to the increase in metabolic rate of *L. shawii* as a result of high water resources of the soil in winter than in summer [37,46]. The Na^+, K^+, Ca^{2+}, Mg^{2+}, N and P contents were significantly increased during dry season in most roots and leaves of the studied plants as *L. europaeum* [21]. The mineral uptake can decrease when stress intensity be increased [47], where the mineral contents of plant parts may be controlled by stress and plant species differ in content of the minerals as well as the reactions under adverse conditions in the same area [48]. Also, the mineral concentrations in tissues of plant species are positively correlated with its habitats [49].

It is noted that some minerals in plant are reduced due to other minerals; the reduction of the N concentration attributed to the Cl^- and NO_3^- antagonism [50], decline of P due to com-petition between Cl^- and $H_2PO_4^-$ [51], the decrease of K due to the existence of competition effects between Na^+ and K^+ ions which most likely share the same transport sys-tem [52] and The rate of Mg^{2+} uptake can be strongly depressed by other cations, such as K^+ and Ca^{2+} [53]. Saline conditions induce an increase in Cl^- and Na^+ concentrations in the roots and leaves. At high concentrations, Na^+ and Cl^- become toxic and cause morphological, physiological, biochemical and molecular disturbances in most plants [54], and thus, K^+ ions are essential for reducing the uptake of Na^+. Under these conditions, the tolerance to salinity in each species is triggered through different strategies such as ions accumulation at root level, shedding of dry leaves, salt secretion and succulence in the different organs [7]. Therefore, Na^+ and K^+ concentrations and ion balance play important roles in plant salt tolerance [55].

3.2.2 Protein Gel Electrophoretic

SDS-PAGE patterns of molecular weight for protein patterns of *L. shawii* leaves under different habitat conditions are given in Table 4 and Fig. 1. Wadi El-Bagha was characterized by the presence of the higher number of bands than in Wadi Hashem during the both seasons. The band No. 1 had recorded the highest molecular mass (148.3 kD) of *L. shawii* in Wadi El-Bagha during autumn. While, the lowest molecular weight had in band No. 27 (10.5 kD) at Wadi Hashem and Wadi El-Bagha during spring and autumn seasons, respectively, this indicates that different molecular weights. Our results are related to the findings of El- Ghamery et al. [56] reported that, the presence of different molecular weights of *L. shawii* leaves during different studied localities in Egypt. The high molecular weight proteins can be involved in lowering Na^+ influx, thus increasing tolerance to salt stress [57]. The leaves of *L. shawii* were characterized by the presence of twelve unique and fifteen polymorphic bands. Four unique bands were found in Wadi Hashem during autumn season with bands No. 3, 9, 11 and 18, while, eight unique bands in Wadi El Bagha during autumn (bands No. 1, 8, 10 and 12) and spring (bands No. 2, 4, 14 and 15) seasons. It observed that the unique protein bands with high molecular weight occur in Wadi El-Bagha during autumn and spring seasons. El-Absy [20] and Abdel Kawy [21] reported that the appearance of most proteins that have specific low molecular weights, which are unique for each plant.

The two persistent bands appeared in both seasons under two studied locations with molecular masses of 35.3 and 29.5 kD. Four protein bands of 125.7, 109.9, 32.7 and 10.5 kD were characterizing Wadi Hashem at spring season and Wadi El-Bagha at autumn season. Another two protein bands having Mwt. of 40.6 and 27.4 kD. appeared in two locations at spring season and Wadi El-Bagha at both seasons, respectively. In addition another two protein bands of Mwt. 102.6 and 56.2 kD. appeared in Wadi Hashem during the both season and in Wadi El-Bagha during spring season as well as another three of Mwt of 46.5, 25.3 and 15.2 kD. appeared in Wadi El-Bagha during the both seasons and in Wadi Hashem during autumn season. A two protein bands of Mwt. 42.7 and 38.4 kD have been formed during the both season in Wadi El-Bagha and during spring season in Wadi Hashem. El-Absy [20] mentioned that the high molecular protein bands (between 90 and 100 kD) have been synthezied in *Nitraria retusa* in most sites in dry and wet seasons and in *Arthrocnemum macrostachyum* in two sites during the wet season. El- Ghamery et al. [56] stated that *L. shawii* showed two specific bands with molecular weight 70 and 54 kDs during different studied localities. Our results indicating that the two studied locations and seasons could be control the appearance and disappearance of protein bands of *L. shawii* leaves. A significant relationship was found between the cultivation year and the percentage of unextractable polymeric protein in the total polymeric protein [58].

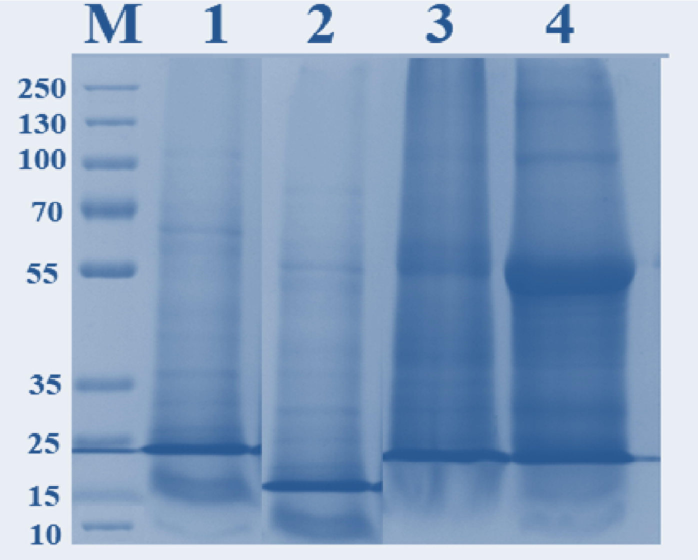

Fig. 1. The produced protein patterns of *L. shawii* leaves under different habitat conditions using SDS-PAGE technique. M: Standard protein marker, 1: Wadi Hashem at autumn, 2: Wadi Hashem at spring, 3: Wadi El-Bagha at autumn, 4: Wadi El-Bagha at spring

Table 3. ANOVA and mean for chemical compositions concentrations (%) of *L. showii* at three sites in spring (Spr.) and autumn (Aut.) seasons during the two studied locations

Locations	**Parameters**	**Na^+**		**K^+**		**Ca^{2+}**		**Mg^{2+}**		**N**		**P**	
	Seasons **Sites**	**Spr.**	**Aut.**	**Spr.**	**Aut.**	**Spr.**	**Aut.**	**Spr.**	**Aut.**	**Spr.**	**Aut.**	**Spr.**	**Aut.**
Wadi El-Bagha	Up-Stream	0.86	1.12	1.91	2.16	0.43	1.81	3.20	2.33	2.82	3.41	1.21	1.90
	Mid-Stream	1.20	1.37	2.13	1.89	0.63	1.92	3.51	4.11	2.91	3.81	2.72	2.91
	Down-Stream	0.31	1.99	0.51	0.32	0.43	0.99	3.34	4.93	1.44	0.99	3.10	2.11
Wadi Hashem	Up-Stream	3.22	2.43	2.30	3.22	4.51	2.31	2.91	1.14	1.40	1.99	0.90	1.20
	Mid-Stream	2.62	3.41	2.45	3.31	5.21	4.11	3.12	2.31	2.30	2.80	1.20	1.90
	Down-Stream	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
L.S.D at		0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01
Locations		*	**	*	**	*	**	*	**	*	**	*	**
Seasons		*	**	*	**	NS	NS	*	**	*	**	*	**
Sites		*	**	*	**	*	**	*	**	*	**	*	**
Locations x Seasons		*	**	*	**	*	**	*	**	NS	NS	*	**
Locations x Sites		*	**	*	**	*	**	*	**	*	**	*	**
Seasons x Sites		*	**	*	**	*	**	*	**	*	**	*	**
Locations x seasons x Sites		*	**	*	**	*	**	*	**	*	**	*	**

** and **: Significant at 0.05 and 0.01 levels of probability, respectively*

Table 4. Changes in produced protein bands of *L. shawii* leaves under different habitats conditions using SDS-PAGE

Marker M.W (kD)	Bands No.	RF	M.W (kD)	Wadi Hashem		Wadi El-Bagha		Polymorphism
				Autumn	Spring	Autumn	Spring	
250	1	0.32	148.3	–	–	+	–	Unique
	2	0.35	146.5	–	–	–	+	Unique
	3	0.39	139.1	+	–	–	–	Unique
	4	0.45	138.4	–	–	–	+	Unique
130	5	0.52	125.7	–	+	+	–	–
	6	0.59	109.9	–	+	+	–	–
	7	061	102.6	+	+	–	+	–
100	8	0.62	83.8	–	–	+	–	Unique
	9	0.64	71.4	+	–	–	–	Unique
70	10	0.67	67.8	–	–	+	–	Unique
	11	0.68	62.1	+	–	–	–	Unique
	12	0.68	60.5	–	–	+	–	Unique
55	13	0.69	56.2	+	+	–	+	–
	14	0.72	52.3	–	–	–	+	Unique
	15	0.74	48.8	–	–	–	+	Unique
	16	0.77	46.5	+	–	+	+	–
	17	0.78	42.7	–	+	+	+	–
	18	0.79	41.9	+	–	–	–	Unique
	19	0.81	40.6	–	+	–	+	–
	20	0.83	38.4	–	+	+	+	–
35	21	0.83	35.3	+	+	+	+	–
	22	0.85	32.7	–	+	+	–	–
	23	0.89	29.5	+	+	+	+	–
	24	0.89	27.4	–	–	+	+	–
25	25	0.91	25.3	+	–	+	+	–
15	26	0.93	15.2	+	–	+	+	–
10	27	0.97	10.5	–	+	+	–	–
		Sum	+	11	11	16	15	
			-	16	16	11	12	

kD: kilo Dalton, (+): present, (–): absent

This variation of the protein pattern in *L. shawii* could be an evidence of the adaptation of the plant to different stresses. This result is similar to the findings of El-Absy [20] and Abdel Kawy [21] on *L. europaeum* and other studied plants in studied regions during the wet and dry seasons. Many molecular chaperones being stress proteins, which function as key components contributing to cellular homeostasis in both optimal and adverse growth conditions [59].

In order to determine the differences among protein bands and determination of the protein bands far or nearness of *L. shawii* leaves under different habitat conditions, the cluster analysis was applied to place the similar protein bands in one group. In Fig. 2, the cluster analysis of protein bands resulted into two group's i.e., unique bands (A) and polymorphic bands (B). The group A divided into three clusters i.e., the cluster C1 (bands No. 1, 8, 10 and 12), the cluster C2 (bands No. 3, 9, 11 and 18) and the cluster C3 (bands No. 2, 4, 14 and 15). While, the group B comprised of four clusters i.e., the cluster C4 (bands No. 19, 17 and 20), the cluster C5 (bands No. 7, 13, 21 and 23), the cluster C6 (bands No. 16, 25, 26 and 24) and the cluster C7 (bands No. 5, 6, 22 and 27). The tree diagram detected minimum distance or dissimilarity between the protein bands of the clusters inside each group. Each cluster from C1, C2, C3 and C7 clusters contained protein bands that were highly similar, while each cluster from the other clusters were highly or moderately similar in protein bands under the two studied locations during autumn and spring seasons. On the other hand, the highest distances were found of the protein bands among the clusters in the two groups A and B. These results indicating differences among protein patterns of *L. shawii* leaves under the two studied locations during autumn and spring seasons. The highest number of protein bands present in five clusters (C1, C4, C5, C6 and C7) was found in Wadi El-Bagha at autumn season. While, the bands of C4, C5 and C6 in Wadi El-Bagha at spring season and the bands of C2, C5 and C6 as well as the bands of C4, C5 and C7 in Wadi Hashem

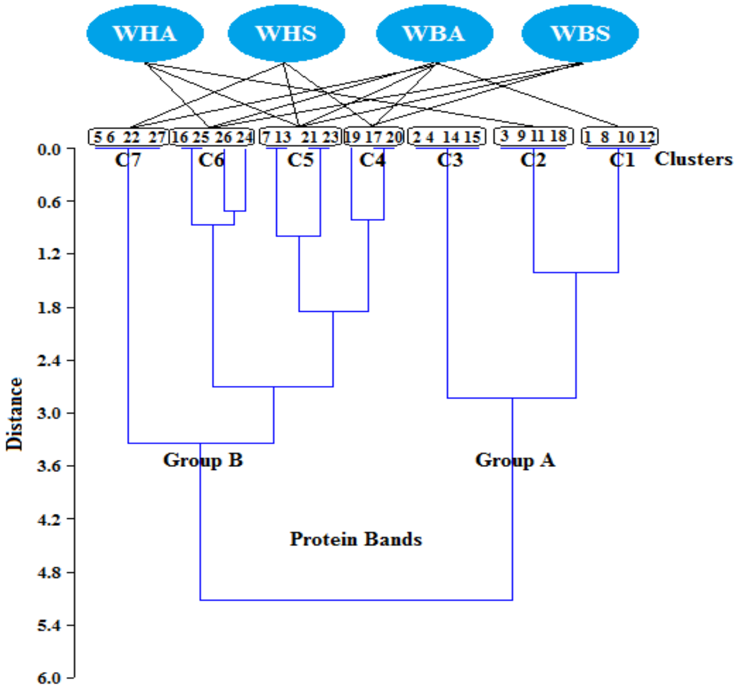

Fig. 2. Dendrogram showing hierarchical classification of 27 protein bands based on the two studied locations during autumn and spring seasons are using Ward method. WHA and WHS: Wadi Hashem at autumn and spring seasons, respectively; WBA and WBS: Wadi El-Bagha at autumn and spring seasons, respectively; C1, C2, C3, C4, C5, C6 and C7: number of clusters

at autumn and spring seasons, respectively were observed. These results show that there is a significant effect of environments (locations or seasons) in controlling the expression of protein patterns of *L. shawii* leaves. Thus, the protein patterns may be considered as key genetic markers of salinity stress tolerance in *L. showii leaves* during Wadi El-Bagha at autumn season, which could be partially attributed to the increased synthesis of new protein molecules under drought or salinity stress to make adaptation [21].

The protein concentration and most of protein components are influenced to a large extent by genetic background of cultivar and environmental factors such as nitrogen and water access and temperature conditions [58,60]. Also, Intrinsic factors as adaptability to environmental change affect their protein surface properties [61]. In response to drought, osmotic and oxidative stresses plants always accumulating heat shock proteins [62]. In different species of plants, reduction in the proteins level that are affected by salinity, have been attributed to the reduction in the production of proteins and enzymes which interfere the production of amino acids and proteins [63].

4. CONCLUSION

By studying the soil and plant analysis of *L. showii* can conclude that significant differences were observed represent genuine environmental variations on mechanical properties and chemical analysis of the soil (depths, sites and locations and interactions them) as well as mineral contents (seasons, sites and locations and their interactions). Most studied elements of *L. showii* were greater in autumn than in spring during the two studied locations. Using SDS-PAGE technique, the protein bands from 1 (148.3 kD) to 27 (10.5 kD) exhibited different molecular weights between the two studied location during both seasons. Highest number of bands and unique high molecular weights proteins had observed in Wadi El-Bagha during the both seasons. Therefore, during these habitats these patterns reflect variations of behavior and adaptation of *L. shawii* under stress conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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