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Seed salt-soluble protein expression as marker of local *Medicago ciliaris* populations adapted to highly salted region from Algeria (Oran Great Sebkha)

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The present study estimated the diversity of seed storage salt-soluble proteins of Algerian populations of *Medicago ciliaris* species, for understanding biochemical and molecular features involved in response during plant stress adaptation. Eleven patterns of *M. ciliaris* populations with two moderate sensitive ecotypes to NaCl, two reference genotypes and seven prospecting populations near and far from a strongly salted area (Sebkha of Oran) were investigated by one dimensional electrophoresis SDS-PAGE. The results show that the proteins profiles were very similar with 10 to 12 bands profiles about all populations, after electrophoresis in denaturing conditions; but an 80 kDa band was visible only on population from Sebkha origin, where the salt content in the soil is the highest one. It appears that, this globulin protein is related to salt tolerance and could be used as SDS-AGE markers for differentiating between tolerating and sensible *M. ciliaris* populations to salt stress.

Key words: Oran Great Sebkha, annual *Medicago ciliaris*, seed storage salt-soluble proteins, sodium dodecyl sulfate- poly acrylamide gel electrophoresis (SDS-PAGE) markers.

INTRODUCTION

The Oran Great Sebkha is formed by a slender stratum of water devoid of vegetation inside. It is located at 35°32'N, 00°48'E. Lands bordering this site are occupied by private grounds, used for farming. The edges of salty lake are used by breeders for the pasture. Before, Sebkha which was not the object of study on vegetation does not seem to contain a remarkable flora. It remains a halophile vegetation compound of *Sueada sp.*, *Juncus sp.* and small bundles of *Chamaeropsis humilis*, and some rare specimens of tamarisk at the level of bands (Moussa and Saint-Martin, 2011). Presently, observation on the vegetal species is enabling the drawing of the map of vegetation growing around the Sebkha and shows the relationship with salinity fluctuation. Annual medics (*Medicago*

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Abbreviations: MW, Molecular weight; **HCA,** hierarchical cluster analysis**; Rf,** relative mobility; **SDS-PAGE,** sodium dodecyl sulfate- poly acrylamide gel electrophoresis; **UPGMA,** unweighted pair group method with arithmetic means.

Table 1. Name, origin, experimental code and genotype of the different studied populations of *M. ciliaris.*

Pop	Experimental code and genotype name	Province and Origin	Latitude	Longitude	Altitude (m)
FA pop	P1: *M. ciliaris modj1*	Mascara Algeria	35°33'24.29''N	0°72'.86E	689
	P2: *M. ciliaris modj2*	Mascara Algeria	35°24'0.64''N	0°7'10.06E	600
	P3: *M.* ciliaris *modj3*	Bredeah Algeria	35°34'60''N	0°51'0''W	110
	P7: *M. ciliaris modj7*	Ain-Tassa Algeria	35°37'22.00''N	0°55'36.25''W	300
NE pop	P4:*M. ciliaris modj4*	Oran Great Sebkha Algeria	35°33'28.88''N	0°50'33.95''W	81
	P5:*M. ciliaris modj5*		35°33'26.83''N	0°50'32.63''W	84
	P6:*M. ciliaris modj6*		35°33'58.73''N	0°50'28.00''W	83
Ref pop	P8:*M. ciliaris 252*	ITGC Algeria	3501N	0018W	470
	P9:*M. ciliaris 255*	ITGC Algeria	3501N	0018W	470
	P10:*M. intertexta.ciliaris IG54229*	Lebanon ICARDA Syria	33 52N	3601E	1000
	P11:*M. intertexta.ciliaris IG54230*	Lebanon ICARDA Syria	33 52N	3601E	1000

spp.) are predominantly selfing annual plant of the Mediterranean region (Lesins and Lesins, 1979). They are winter annual legumes which indicate a strong ability to adapt to local environments. These species are of special interest, since they form symbioses with nitrogen-fixing bacteria and are, therefore, excellent candidates for the low-input improvement of marginal or degraded lands with low fertility. They are critical components of natural ecosystems and agriculture and have recently been the subject of several studies highlighting their benefits and responses to cultivation in saline conditions (Abdelly et al., 2011). Among these legume species, *M. ciliaris (L.)* appeared to be more salt tolerant within a collection that included *Medicago polymorpha*, *Medicago truncatula* and *Medicago minima*, since it maintained its biomass productivity when growing in 100 mM NaCl (Abdelly et al., 1995). *M. ciliaris* species would have strong potential to be used in the reclamation of areas, such as Sebkha edges. Based on the fact that they were one of the glycophyte plants that have possibility to grow under this salt condition and were competent to improve the quality and quantity of pasture.

M. ciliaris L. is a diploid species (2n=16) belonging to the section of *Spirocarpos* subsection of *Intertextae* as delimited by Heyn (1963). Understanding the genotypic variation for salt adaptation is a key for developing selection and breeding strategies. The main strategy used over the past few years to improve salt tolerance in legumes has been genotype screening and selection (Cordovilla et al., 1995).

Thus, the objective of many studies was to explore genotypic variation for salt tolerance using many indicators of plant's health. Nevertheless, the seed storage proteins associated with ecological data of this species could be very promising. They are useful tools for a preliminary investigation of genetic diversity of local populations of *M. cilaris L*. In addition, Seed proteins are classified according to their solubilities either as water-soluble albumin, salt-soluble globulin, alcohol-soluble prolamin, and acid-or alkaline-soluble glutelin. Seed storage proteins include mainly globulins in legumes. They are the dominant storage proteins in legume seeds and account for 50 to 90% of seed proteins (Rashidah et al., 2007). The expression of seed storage proteins is under strict developmental regulation and represents a powerful model system to study the regulation of gene expression during plant development (Mehrotra et al., 2009).

Present study deals with comparison of three prospecting populations of *M. ciliaris* growing near the Oran Great sebkha and four others far from it. In this way, we examined the expression of seed salt-soluble proteins from different populations of *M. ciliaris* using the cryo precipitation technique for extraction and SDS-PAGE for separation. This information could then be used to define selection criteria and provide a framework for selecting large numbers of populations that grow in all sides of Sebkha edges.

MATERIALS AND METHODS

Plant material

The material included 11 populations of *M. ciliaris*; tow ecotypes were represented on the moderate sensitive ecotypes to salt stress from ITGC Algeria; seven were prospected near and far from the Great Sebkha of Oran and two reference exotic genotypes (*Intertexta vir. Mcilaris*) were supported from ICARDA, Syria. They are formed in three groups of populations Table 1; faraway population (FA pop), nearest po pulation (NE pop) to Oran Great Sebkha and the reference population (Ref pop).

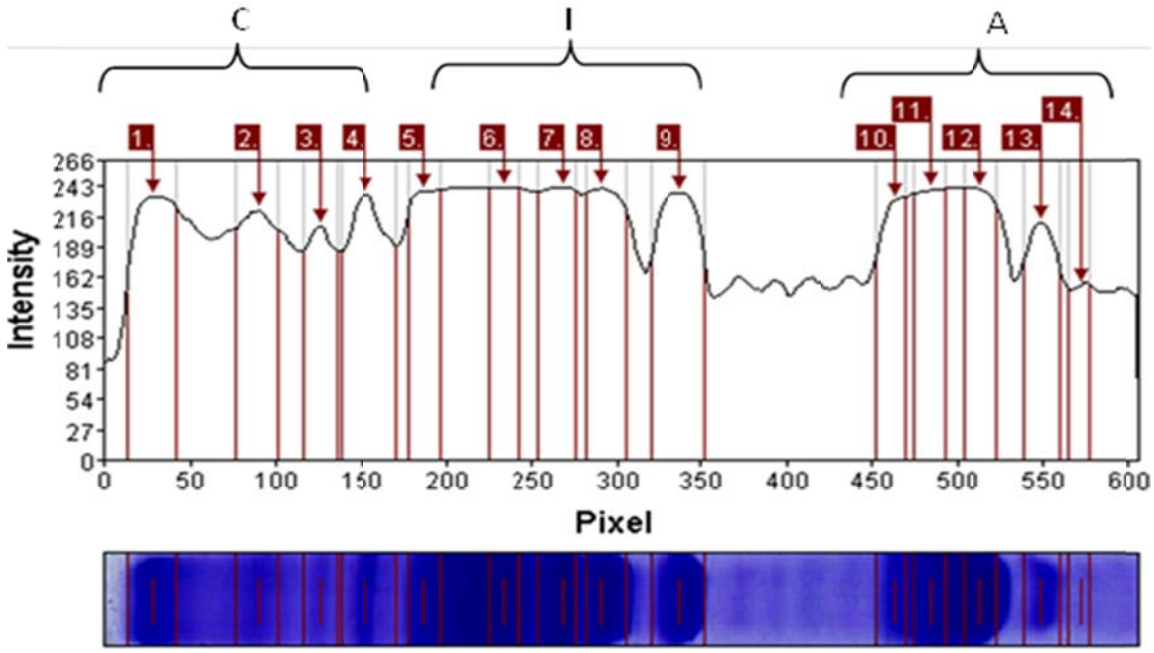

Figure 1. Profile windows content based on software Gel Analyzer V.2010a of the reference genotype P10 (*M. intertexta. ciliaris IG54229)* with the three zones C, cathodic zone; I, intermediary zone and A, anodic zone. 1, 2, 3…14: indicated band number.

Protein extractions

Mature seeds were used for isolation seed salt-soluble proteins. Ten seeds of each population were ground in four volumes of 1-butanol with mortar and pestle to eliminate lipids. Protein fraction was extracted by cryo-precipitation (Anisimova et al., 1991 modified by Fyad-lameche, 1998).

Electrophoretic analysis (SDS-PAGE)

One-dimensional SDS-PAGE (4.5% stacking gel and 13.5% resolving gel) was performed according to Laemmli (1970). The electrophoresis was performed for 4 h at 150 V constant. The gels were stained with Coomasie brilliant blue R250 and then destained in methanol/ acetic acid. After destaining, the electrophoretic bands were clearly visible. The electrophoresis was repeated 4 times. Biolabs Protein Ladder was used as molecular weight marker. It is a mixture of 12 recombinant, highly purified proteins, which resolve into clearly identifiable sharp bands from 10 to 250 kDa (Laemmli, 1970; Sambrook et al., 2001). The 25 and 80 kDa bands have triple the intensity of the other proteins and serve as reference indicators (unpublished results).

Statistical analysis

A comparison of electrophoresis bands was performed based on their thickness, their number and their mobility. The protein ladder is intended for use as a precise size standard when performing SDS-PAGE to calculate the molecular weight of a protein of interest. To avoid any ambiguity, the experience was repeated 4 times and the gels were analyzed using the computer software Gel Analyzer V.2010a. Only the major and clearly bands in the gels were considered for data recording. The data was recorded as presence (1) or absence (0) of protein bands, entered in a binary matrix and then analyzed by statistical procedures. Hierarchical cluster analysis (HCA), similarity and distance matrix were conducted using DendroUPGMA (http://genomes.urv.cat/UPGMA/) applying Jaccard's coefficient with default settings (Garcia-Vallvè et al., 1999).

RESULTS AND DISCUSSION

All profiles were analyzed according software Gel Analyser based on band intensity curves. Using one profile (P11) of the two reference genotypes *M. intertexta vr.ciliaris* (Figure 1), three different zones were recorded (Figures 1 and 2); one is cathodic zone (C), the second is intermediate (I) and the last is anodic zone (A). Each zone contained the major and minor bands. According to the protein ladder, their molecular weights ranged between 60-250, 15-50 and 10-≥15kDa, respectively. In addition, it was observed that protein profiles of most of the populations were the same for the major bands. There are many common bands among populations. But specific bands were also observed, it was indicated by a dark arrow (Figure 2).

The most number of bands belonged to (NEpop) populations with important intensity and profiles with least number of bands with low intensity were related to those of (FApop) distant populations to the Oran Great Sebkha. They presented 15 and 13 bands, respectively. A specific band of 80 kDa with the Rf 0.162 was present in all nearest populations (NEpop) and it was missing in the

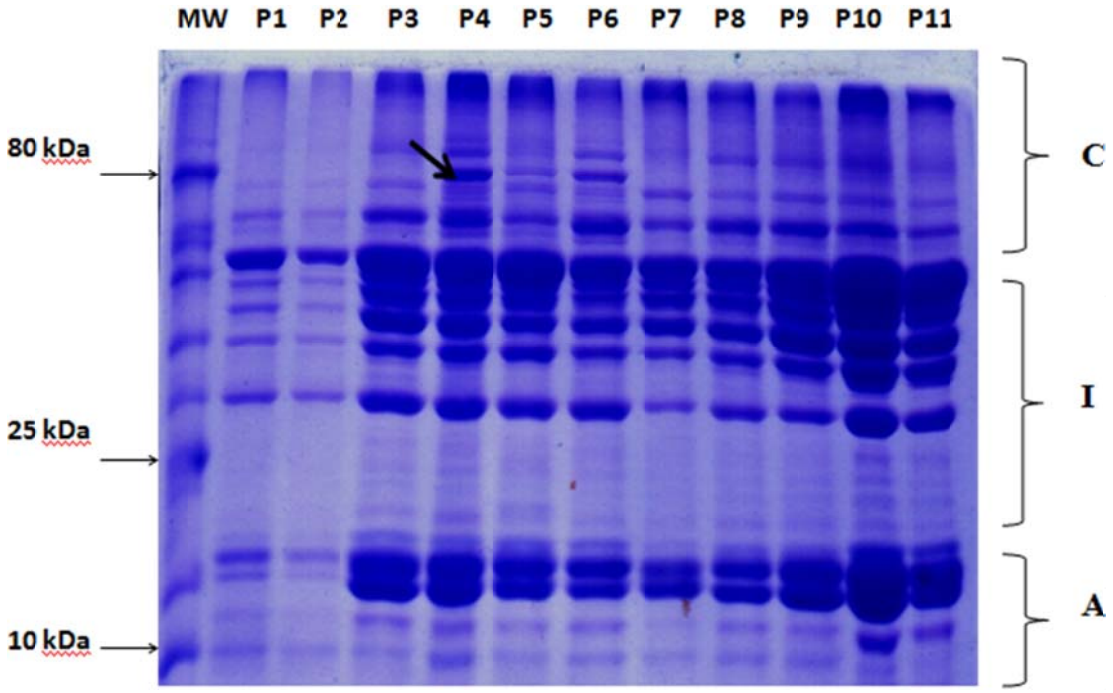

Figure 2. SDS-PAGE of seed salt-soluble proteins of *M. ciliaris* populations with Lanes: MW, Ladder protein (standard protein molecular weight markers); P1, *M. ciliaris modj1;* P2, *M. ciliaris modj2;* P3, *M.ciliaris modj3;* P4, *M. ciliaris modj4;* P5, *M. ciliaris modj5;* P6, *M. ciliaris modj6*; P7, *M. ciliaris modj7;* P8, *M. ciliaris 252;* P9, *M. ciliaris 255;* P10, *M. intertexta. ciliaris IG54229* ;P11, *M. intertexta. ciliaris IG54230 and three zones of migration:* C, cathodic zone; I, intermediary zone; A, anodic zone.

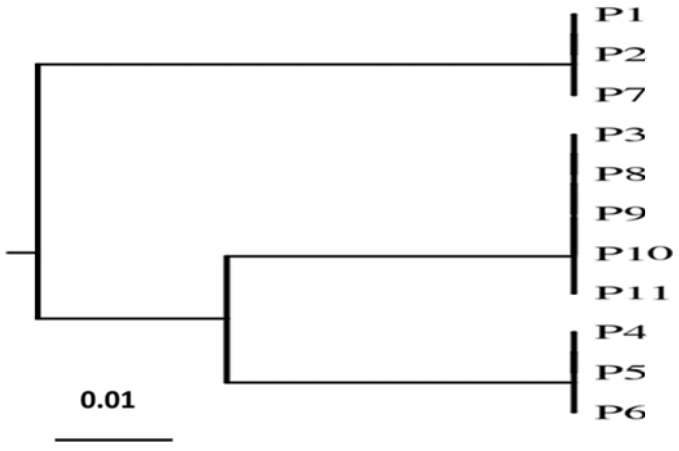

Figure 3. UPGMA dendrogram based on binary matrix of seed salt-soluble protein bands of *M. ciliaris* populations.

distant (FApop) and reference populations (Ref pop). Fareghi et al. (2007) studied the variability between 18 genotypes of Lucerne using SDS-PAGE. They reported 16 bands related to salt-soluble proteins.

Based on cluster analysis by UPGMA method (Figure 3) populations were classified into three groups. The first group consisted of 3 genotypes (P1, P2 and P7), the second group included five genotypes (P3, P8, P9, P10 and P11) and the third group consisted three genotypes (P4, P5, P6). Similarity and distance matrix based on Jaccard coefficient were shown in Tables 2 and 3. The highest similarity and minimum genetic distance belonged to each intra groups with similarity coefficient 1. The lowest similarity and highest genetic distance were observed between first group and third group with similarity coefficient 0.867. They are the distant and close populations to the Oran Great Sebkha, respectively. These two last groups have important similarity with reference group, moderate genetic distance with similarity coefficient 0.933 and 0.929, respectively.

These results confirmed the output dendrogram demonstrated with Phylip format:(((P1:0.000,P2:0.000):0.000,P7:0.000):0.051,(((((P3:0.000,P8:0.000):0.000,P9:0.000):0.000,P10:0.000):0.000,P11:0.000):0.033,((P4:0.000,P5:0.000):0.000,P6:0.000):0.033):0.018); According the output dendrogram, the three groups were clearly separated (Figure 3).

In addition, we observed that the distant populations to Oran Great Sebkha were differed than the tow moderate sensitive ecotypes *M. ciliaris* 252 and 255 by the low intensity of band, although variation was observed in the density or sharpness of a few bands. This indicates that, the salt-soluble protein fractions, such as globulins, should be used for studying the relationship of the seeds

Table 2. Similarity matrix computed with Jaccard coefficient for seed salt-soluble proteins of studied populations of *M. ciliaris.*

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
P1	1	1.000	0.929	0.867	0.867	0.867	1.000	0.929	0.929	0.929	0.929
P2		1	0.929	0.867	0.867	0.867	1.000	0.929	0.929	0.929	0.929
P3			1	0.933	0.933	0.933	0.929	1.000	1.000	1.000	1.000
P4				1	1.000	1.000	0.867	0.933	0.933	0.933	0.933
P5					1	1.000	0.867	0.933	0.933	0.933	0.933
P6						1	0.867	0.933	0.933	0.933	0.933
P7							1	0.929	0.929	0.929	0.929
P8								1	1.000	1.000	1.000
P9									1	1.000	1.000
P10										1	1.000
P11											1

Table 3. Distance matrix based on Jaccard coefficient.

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
P1	0	0.000	0.071	0.133	0.133	0.133	0.000	0.071	0.071	0.071	0.071
P2		0	0.071	0.133	0.133	0.133	0.000	0.071	0.071	0.071	0.071
P3			0	0.067	0.067	0.067	0.071	0.000	0.000	0.000	0.000
P4				0	0.000	0.000	0.133	0.067	0.067	0.067	0.067
P5					0	0.000	0.133	0.067	0.067	0.067	0.067
P6						0	0.133	0.067	0.067	0.067	0.067
P7							0	0.071	0.071	0.071	0.071
P8								0	0.000	0.000	0.000
P9									0	0.000	0.000
P10										0	0.000
P11											0

and their environmental origin. Previously, Hedrick et al. (1976) demonstrated, that the genetic variation between and within populations is considered as a result of the environmental heterogeneity and the action of natural selection. Presently, Lazrek et al. (2009) investigated the genetic diversity of a collection of annual *Medicago* species and found that, it had a correlation with environment salinity of origin of these populations. Recently, Amouri et al. (2014) demonstrated that the tolerant genotype to salt stress of annual *Medicago* represented maximum number of total protein bands and the sensitive corresponded minimum number of polypeptide bands using SDS-PAGE.

Our results concluded that the material prospected near and far from the Oran Great Sebkha exhibited moderate genetic diversity for one dimensional SDS-PAGE. Therefore, 2D-electrophoresis is needed to separate various portions of the gel. This later technique has already been used by Li et al. (1998) and they reported their usefulness. These techniques can be applied to studies of storage proteins in other seeds as well as non-seed storage proteins. Krochko and Bewley (1998) used the two-dimensional electrophoresis to determine the composition of seed storage protein fractions in alfalfa. They found that the major seed storage proteins in alfalfa are medicagin (a legumin-like globulin), alfin (a vicilin-like globulin) and a family of lower molecular weight (albumins). These comprise 30, 10, and 20%, respectively; of the total extractable protein from mature seeds. Kaviani and Kharabian (2008) demonstrated that alterations in salt (fertilizers) levels in soil could change the subunits of seed protein legumes.

In general, seed storage protein profiling based on SDS-PAGE can be employed for various purposes such as varietal identification, biosystematics analysis, determination of phylogenetic relationship between different species, generating pertinent information to complement evaluation and passport data (Sammour, 1991). Our results corroborate these objects because we found that seed protein fractions are power tool to identify the adapted populations to Sebkha edges and we can use this substantial protein fraction as molecular markers.

Furthermore, seed proteins are mainly storage proteins and are not likely to be changed in dry mature seed; their

composition is highly stable and is affected only slightly by environmental conditions or seasonal fluctuations (Ladizinsky and hymowitz, 1979). Previously, numerous studies in the last decade have shown that intrinsic changes in the plant such as chromosomal rearrangements or even doubling of chromosome numbers have no, or very small, effects on seed protein profile. The seed storage proteins are encoded by small fraction of the genes expressed in seeds and their expression is regulated at transcriptional, post-transcriptional, translational and post-translational levels (Mehrotra et al., 2009). A precise knowledge about seed protein genes, their regulatory mechanisms and the proteomic approaches can help us to resolve a part of relationship between grain salt-soluble proteins and the adaptation to salt conditions.

Conclusion

This study demonstrates that populations from Oran Great Sebkha edges seem to express a higher level of protein production than distant p, the more expression of seed salt-soluble proteins. It may be concluded that hybridization between populations from the two groups is suggested to be conducted with the expectation that band 80 KDa and other minor bands might be linked with salt stress adaptation. This would help in planning experiments for marker assisted breeding in *M. ciliaris*. The obtained results also allow us to direct the choice of the sites of collections for the future prospecting and the choice of parents according to the fixed objectives (crossing, selection, gene candidates etc).

Conflict of Interests

The author(s) have not declared any conflict of interest.

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