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Comparison of genetic diversity among *Medicago ciliaris* populations prospected from highly salted region (Oran Great Sebkha) using morphological pod descriptors of IBPGR and SDS-PAGE markers

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Abstract

With the aim of characterizing and evaluating spontaneous *M. ciliaris*species of annual *Medicago* genus which are adapted to salty conditions. A comparison was realized on 11 populations among which seven are prospected near and far from the Great Sebkha of Oran while four others are used as a reference. This study is pressed on two approaches, the one is based on the biometrics of the morphological traits of pods using IBPGR descriptors, and the other one is a biochemical approach using the one-dimensional sodium dodecyl sulfate poly acrylamide gel electrophoresis (SDS-PAGE) of the seed storage proteins. The biometric results showed a higher level of significance ($p < 0.0001$) for the almost characters, but not absolutely significant at ($p > 0.05$) for the number of coils NC, number of seeds per pod (NS) and for pod height (H). Accordingly, the nearest population from the Sebkha has a dwarfish aspect of pods than the others populations. The SDS-PAGE of total and globulin proteins revealed only minor differences between populations in the number, density, and intensity of polypeptide bands. So we found two distinctive polypeptide bands were observed for globulin and total protein gel, respectively; the first has~80kDa and it's specific to the nearest population. The second is missing from it and has ~68 kDa. Itappears that the combination of morphological pod traits and SDS-PAGE markers are useful tools for a preliminary investigation of genetic variation of *Medicago ciliaris*. This study helped us to determine the population that has adapted to salty conditions.

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Introduction

Medicago is recognized as one of the most important genera of pasture plants in the world (Heyen, 1963).By their integration into *ley-farming*, they can play an important role in crop production increase. They are also able to improve pastoral production in semiarid areas (Salhi Hannachi *et al*., 1998). In Algeria, in the zones of arid and semiarid lands, in particular farmland near Great Sebkha of Oran, the problem of the salinity is one of the limiting factors of the vegetable productivity and the agricultural yield (Moulai and Fyad-Lameche, 2014), it causes harmful effects on soils and plants (Djamai *et al*., 2017). But the introduction of the foreign varieties of *Medicago* to the countries of the Maghreb since 1970, with the aim of improving their forage crops revealed the bad adaptation to the local conditions of introducing cultivars (Seklani *et al*., 1995).

In other hand, the *Medicago* genus presented well, particularly in Algeria, constitute an extremely rich and diversified phytogenetic patrimony (Abdelguerfi *et al*., 1988). Among the species of this genus, *M. ciliaris* is predominantly selfing annual plant of the Mediterranean region (Heyen, 1963; Lesins and Lesins, 1979) it is omnipresent in the semiarid regions and supports the pastoral activity. Their remarkable biomasses make it able to be more salt tolerant (Abdelly *et al*., 1995). This species have recently been the subject of several studies highlighting their benefits and responses to cultivation in saline conditions (Abdelly *et al*., 2010).They would have strong potential to be used in the reclamation of areas, such as Sebkha edges. Because they were one of the glycophyte plants that have possibility to grow under this salty condition and were competent to improve the quality and quantity of pasture.

In addition, the fruit of this species, recognized as pod, is then most characteristic part of the plant (Jabri *et al*., 2012. Almost half of species of the genus *Medicago* can identify and characterized based only on their morphological pod appearance (Lesins and Gillies, 1972).

Besides, assessment of genetic diversity based on morphological characters has been often less efficient and biased owing to high environmental interaction (Tripathy *et al*., 2015). The SDS-PAGE of seed storage proteinsis, however, considered as practical, cost effective and reliable method as it is largely independent of environmental fluctuations (Iqbal *et al*., 2005; Dutta and Mallick, 2012). The major seed storage proteins in *Medicago* are a 7S vicilin-like globulin (alfin), an 11S legumin-like globulin (medicagin) and a 2S albumin (LMW) (Krochko and Bewley, 2000). These proteins comprise 10%, 30% and 20%, respectively, of the total accumulated protein in mature seeds of *M. sativa* (Krochko *et al*., 1994). Thus, globulin storage proteins provide an excellent model for the study of the plant gene regulatory mechanisms (Milisavljevic *et al*., 2004). In other hand, the use of these protein markers in combination with morphological traits of pods can constitute an important alternative to describe and compare the genetic variability among a set of prospected populations of *M. ciliaris* witch have a good adaptation to constraint environments such as Sebkha edges.

Materiel and methods

Plant material

Eleven of the self-pollinating annual species *M. ciliaris* populations from Genetic and Plant Breeding Laboratory, Oran University were chosen (Table 1) based on their geographical differences. They are formed three groups of populations; faraway population (FA pop), nearest population (NE pop) to Oran Great Sebkha and the reference population (Ref pop).

Survey area

Seven of these populations were collected from different regions near and far from Oran Great Sebkha. This salted region is located at the North of Algeria (Fig.1). With Latitude: 35°31'9.65"and Longitude: -0°49'11.75". The estimate terrain elevation above sea level is 83 meters. The lake basin is supplied by sodium chloride. Moussa *et al* (2014) recognized major group's plant species proliferating in the peripheral Sebkha areas.

Apart from the high parts and Murdjadjo Tessala, other parts of the Sebkha edges are occupied by salt-tolerant species. The plain of Sabkha itself is completely bare.

Data collection of morphological traits

Data were recorded for morphological traits using the international norms of IBPGR (1991) concerning the genus *Medicago*. These parameters were measured on each pod individually on a sample of 10 pods taken randomly from each population.

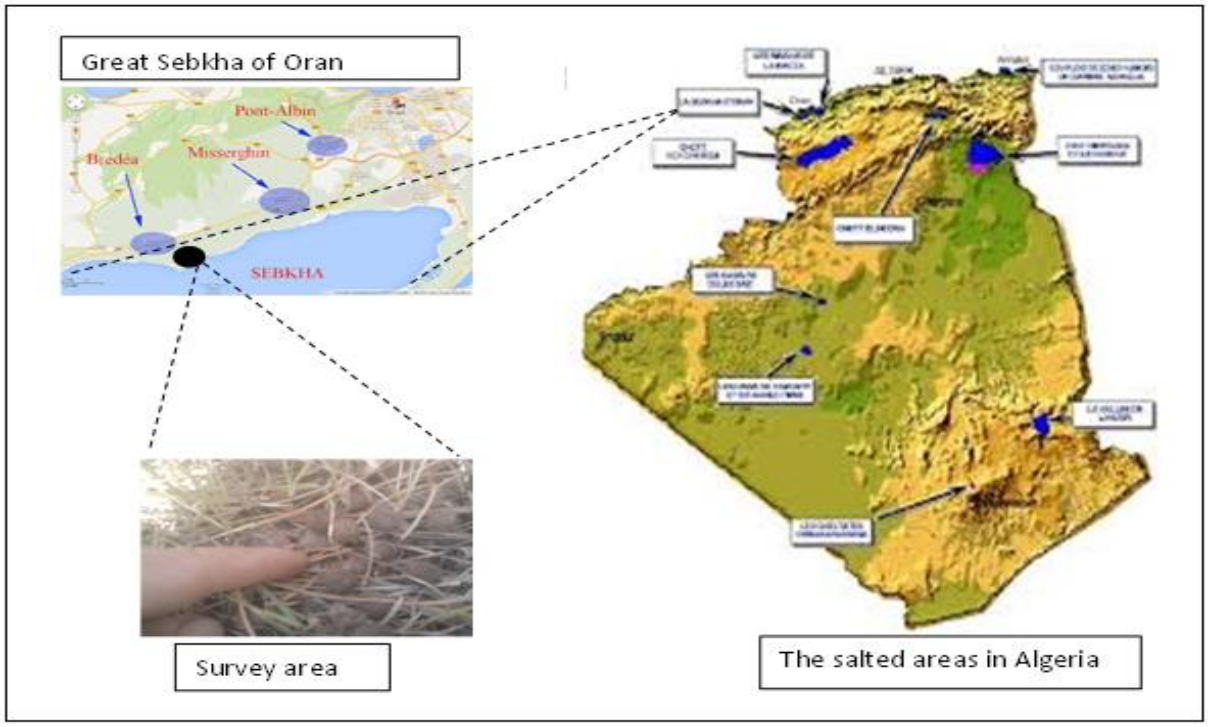

Fig. 1. Map of Algeria showing the location of survey area with the black circle.

Eight parameters were scored on pods of the eleven populations of *M. ciliaris*: PW Pod weight(mg); NC Number of coils per pod(number); D pod diameter(mm); H pod height(mm); PTL pod total length (mm); Ns number of spines per pod (number); NS number of seeds per pod and D/H ratio. For PTL is added as a new measured parameter.

The genotypic variance absolute is estimated by the method used by Kauland Bahn (1974).

$$Vg = \frac{\text{Mean square population} - \text{Mean square error}}{\text{Number of replicate}}$$

And the phenotypic variance is equal to the sum of genotypic variance and residual variance

$$Vp = Vg + Ve$$

The coefficients of genetic variations (C*vg*) are obtained by dividing the square root of the genotypic variance (*Vg*) by the average of the population (μ) and expressed in percentage: $Cvg = \frac{\sqrt{Vg}}{\mu}$

The coefficients of phenotypic variations (C*vp*) are the ratio of the standard deviation *S*of the mean $\bar{X}$. Plus the value of the coefficient of variation is highest, the greater the dispersion around the mean is large. It is usually expressed as a percentage and without unity

$Cvp = \frac{S}{\bar{X}} * \ 100\%$

Seed storage protein extraction

About 10 dry mature seeds for each population. The extraction is performed by two different methods. The first concerning the total proteins, they were extracted according to the method of Leammli (1970) using SDS 2% detergent. The second extraction is specific for globulins according to the protocol (Anisimova *et al*., 1991modified by Fyad-Lameche, 1998).

For each method, three replicates are performed separately. This makes a grand total of 60 seeds per population.

D electrophoresis

The same groups of population used in the biometric analysis were the subject to 1-D electrophoresis with the large tank. The gels are subjected to a 25 mA current for 30 minutes and a voltage of 150 V and 30 mA until the bromophenol blue output. For total proteins and globulins, the separation gels were10% and 13.5%, respectively, with the same stacking gel of 4.5% of acrylamide.

The gels are then stained blue in a solution containing Coomassie R250, methanol and acetic acid and water. Discoloration has the same composition without the Coomassie.

The Protein Marker VI used as molecular weight; it is a three-color protein standard with 12 pre stained proteins covering a wide range molecular weights from 11 to 245 kDa. Proteins are covalently coupled with a blue chromophore except for two reference bands (one green and one red band at 25 kDa and 75 kDa, respectively) when separated on SDS-PAGE (Tris-glycine buffer). This protein ladder is designed for monitoring protein separation during SDS-polyacrylamide gel electrophoresis.

SDS-PAGE analysis

A comparison of electrophoresis bands was performed based on their thickness, their number and their mobility. To avoid any ambiguity, the experience was repeated 3 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.

Statistical analysis

Data of various studied parameters were processed based on Tanagra software (varsion,1.4.50) (Rakotomalala,2005) and Microsoft Office Excel (varsion;2010).

The variance analysis and means comparisons were performed by (one-way ANOVA) and Newman-keuls tests respectively.

Results

Analysis of morphological pod traits

Analysis of variance with one-way ANOVA applied on morphological traits showed a highly significant effect of population at ($p<0.001$) for almost characters with the highest level (Table 2). Except for the following parameter NC and NS, that, they corresponded the number of coils per pods and the number of seeds per pod respectively.

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

Species	Population groups	Experimental code and genotype name	Collection	Province and Origin	Latitude	Longitude	Altitude (m)
M. ciliaris	FA pop*	P1: unknown	New collection	Mascara Algeria	35°33'24.29"N	0°72'.86E	689
		P2: unknown		Mascara Algeria	35°24'0.64"N	0°7'10.06E	600
		P3: unknown		Bredeah Algeria	35°34'60"N	0°51'0"W	110
		P7: unknown		Ain-Tassa Algeria	35°37'22.00"N	0°55'36.25"W	300
M. ciliaris	NE pop*	P4: unknown	New collection	Oran Great Sebkha	35°33'28.88"N	0°50'33.95"W	81
		P5: unknown		Algeria	35°33'26.83"N	0°50'32.63"W	84
		P6: unknown			35°33'58.73"N	0°50'28.00"W	83
M. ciliaris	Ref pop*	P8:*cil252*	INA Algeria		3501N	0018W	470
M. ciliaris		P9: *cil255*	INA Algeria		-	-	-
M. intertexta.v.ciliaria		P10: *IG54229*	ICARDA Syria	Lebanon	33 52N	3601E	1000
M. intertexta.v.ciliaris		P11: *IG54230*	ICARDA Syria	Lebanon	-	-	-

FApop*: populations of *M. ciliaris* with unknown genotype name, which prospected far from the Oran Great Sebkha.
NEpop*: populations of *M. ciliaris* with unknown genotype name, which prospected near the Oran Great Sebkha.
Ref pop*: populations of *M. cliaris* and *M. intrtexta* used as reference populations.

They are not significant at ($p>0.05$) for this study. However, there is no difference between the three groups of populations of these parameters (Table 2). In addition, the Newman-Keuls test at ($p <0.05$) of this parameter make all groups of the population in one homogenous group. But the same test makes often (P4, P5and P6) for almost parameters in the same homogenous group Nepop.

Table 3. Evaluations of genetic parameters in three groups of *M. ciliaris* populations.

PG	MT	*Vp*	*Vg*	*Ve*	*Cvg*(%)	*Cvp*(%)	μ
FA pop	PW*(mg)	0.004	0.003	0.0013	17.8052	21.7389	0.294 mg
	NC*(number)	0.687	0.549	0.1371	9.3533	10.4552	7.925
	D*(mm)	1.362	0.900	0.4622	8.0801	9.9400	11.743 mm
	H*(mm)	0.590	0.237	0.3536	4.2657	6.7354	11.409 mm
	PTL(mm)	2.749	1.828	0.9205	6.1914	7.5919	21.837 mm
	Ns(number)	64.035	3.097	60.9381	1.4265	6.4861	123.375
	NS*(number)	1.717	0.031	1.6863	1.9500	14.5203	9.025
	D/H ratio	0.034	0.002	0.0322	4.4594	18.4515	1.003
NE pop	PW*(mg)	0.002	0.000	0.0020	0.0000	18.5035	0.243 mg
	NC*(number)	0.944	0.375	0.5689	8.0910	12.8383	7.567
	D*(mm)	1.229	0.132	1.0969	3.5538	10.8287	10.239 mm
	H*(mm)	1.233	0.114	1.1185	3.1449	10.3266	10.752 mm
	PTL(mm)	4.116	0.665	3.4510	4.3456	10.8107	18.767 mm
	Ns(number)	35.206	4.235	30.9709	1.8874	5.4419	109.033
	NS*(number)	3.316	0.088	3.2283	3.3540	20.6153	8.833
	D/H ratio	0.013	0.006	0.0075	8.0087	12.0659	0.958
Ref pop	PW*(mg)	0.007	0.006	0.0011	30.1436	32.9248	0.256 mg
	NC*(number)	0.574	0.075	0.4994	3.5110	9.7162	7.800
	D*(mm)	3.222	3.370	-0.1483	17.6616	17.2687	10.394 mm
	H*(mm)	0.847	0.553	0.2940	6.5187	8.0682	11.405 mm
	PTL(mm)	6.215	4.686	1.5298	10.7961	12.4343	20.050 mm
	Ns(number)	109.003	106.639	2.3632	9.5308	9.6358	108.350
	NS*(number)	1.574	1.118	0.4559	12.2580	14.5447	8.625
	D/H ratio	0.014	0.010	0.0030	10.6919	12.8028	0.958

PG: population groups; MT: morphological traits; *Vp*: phenotypic variance; *Vg* genotypic variance; *Ve*: residual variance; *Cvg: coefficient of* genetic variation; *Cvp:* coefficient of phenotypic variation.

In Table 3, the mean values (μ) for all morphological traits of NEpop group appear lower than those values of FApop and Ref popgroups. For example the average value of the character H for NEpop; 10.7mm is lower than those in the other two groups together 11.4 mm and the value of the total length of the pod (PTL)for NEpop group represents 18.7 mm which is inferior than FApop and Refpop groups, that are 21.8 and 20 mm respectively. For the three population groups, the character's weight of the pod (PW) and the ratio D/H values of the genotypic variance are almost zero.

Table 4. Correlation matrix of the coefficients between the different characters.

	PW	NC	D	H	PTL	Ns	NS	D/H
PW		+0.33**	+0.83**	+0.68**	+0.73**	+0.56**	$+0.14^{NS}$	+0.38**
NC			+0.21*	$+0.06^{NS}$	+0.58**	$+0.08^{NS}$	$+0.09^{NS}$	$+0.13^{NS}$
D				+0.51**	+0.67**	+0.68**	+0.33**	+0.58**
H					+0.52**	+0.44**	-0.13^{NS}	-0.06^{NS}
PTL						+0.61**	+0.24*	+0.33**
Ns							+0.28**	+0.37**
NS								+0.31**
D/H								

Referring to previous tables for the abbreviation of characters.
*: significant at p=0.05
**: significant at p=0.01
NS: not significant at p=0.05

The correlations between 8 studied morphological traits in all populations are highly significant (Table 4) for almost but only a few values will be not significant. Correlation of the pod weight with the number of coils per pod, the diameter and height of the pod, the total length pod, number of spines per pod and the ratio D/H was positive and significant. Diameter and height were highly correlated with pod weight 0.83 and 0.68 respectively.

Table 5. Number of total protein bands obtained by 10% acrylamide gel with molecular weight (MW), mobility relative Rf range and specific band according software Gel Analyser10a.

Samples	Number of all bands	Range of MW (kDa)	Range of Rfs	Specific bands (kDa)
Proteins Marker VI	12	11-245	0.062-0.969	75 and 25
P1	44	22-307	0.01-0.986	~68
P2	46	11-307	0.01-0.976	~68
P3	45	11-305	0.014-0.976	~68
P4	35	11-305	0.014-0.976	no
P5	34	11-324	0.003-0.976	no
P6	36	11-324	0.003-0.976	no
P7	37	11-317	0.007-0.976	~68
P8	37	11-324	0.003-0.976	~68
P9	37	11-324	0.003-0.976	~68
P10	36	11-312	0.01-0.976	~68
P11	37	11-317	0.007-0.976	~68

SDS-PAGE analysis of seed storage protein

The seed protein profiles analyzed in the present study showed in general a high degree of similarity among different groups *of M. ciliaris* population in the qualitative and quantitative expression of seed proteins suggests a high degree of uniformity in the seed physiology and genetics of *M. ciliaris*. Three distinct zones were visible on the right of each gel; cathodic (C), intermediate (I) and anodic (A) zone. When compared with the broad-range protein Marker VI applied along with the samples (in the left of each gel) their molecular weights ranged between 245-100, 100-25 and 25-11 kDa, respectively for the total protein gel.

But for the globulins gel, they ranged between 245-63, 63-25 and 20-11 kDa (Fig.2 and Fig. 3).

Differences in polypeptide bands indicate the variability between population groups.

Table 6. Number of globulin bands obtained by 13.5% acrylamide gel with molecular weight (MW), mobility relative Rf range and specific band according software Gel Analyser10a.

Samples	Number of all bands	Range of MW (kDa)	Range of Rfs	Specific bands (kDa)
Proteins Marker	12	11-245	0.029-0.908	75 and 25
P1	15	7-144	0.034-0.975	no
P2	15	12-113	0.112-0.813	no
P3	15	12-110	0.119-0.816	no
P4	18	8-135	0.054-0.953	~80
P5	17	11-109	0.121-0.861	~80
P6	18	12-134	0.056-0.822	~80
P7	18	7-157	0.007-0.987	no
P8	17	11-157	0.007-0.856	no
P9	16	10-156	0.009-0.885	no
P10	16	11-156	0.009-0.856	no
P11	17	12-155	0.011-0.816	no

There are many common bands in both gels among population groups. For the total proteins, the number of bands belonged to P2 and the least number related to P5 with 46 and 35 bands respectively (Table5). But from the Table6, the most and the least numbers of globulin bands are 15and18 respectively. Two specific bands were also observed and they are indicated by dark arrow in both gels (Fig.2 and 3).

Discussion

These highly significant differences of ANOVA indicate the presence of a genetic variability important for studying populations. This result is partially corroborated with that reported by (Heyen, 1963) in the last decades, and actually by Jabri *et al.* (2012).

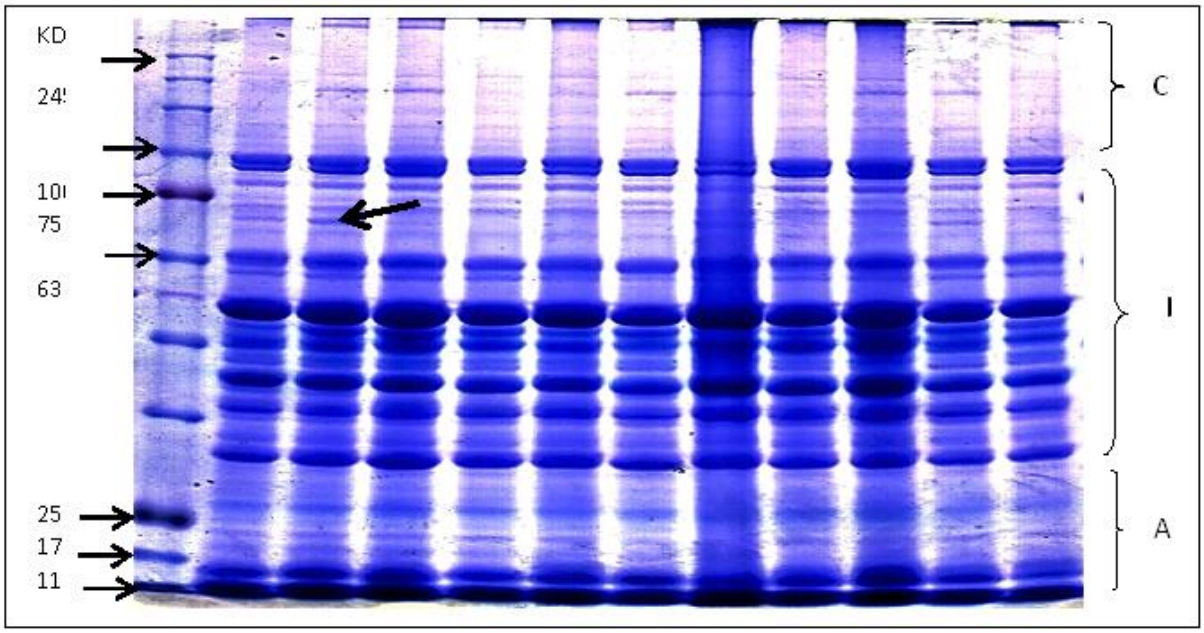

Fig. 2. The SDS-PAGE of total proteins with 10% acrylamide gel from mature seeds of 11*M.ciliaris*. MW: molecular weight; 1, 2...11; corresponded population P1, P2....P11 respectively.

These authors mentioned that the distinction between species of the genus *Medicago* was based mainly on pod morphology. However, the taxonomic studies on the genus *Medicago* in Egypt reported by Ahmed and Atia (1994) demonstrate that, the occurrence of intermediate forms and considerable variation in pod characters make difficult the identification and classification of this genus.

Previous work showed that others parameters like precocity, vegetative development and seed production contribute to the distribution of the diversity (Salhi Hannachi, 1996). In *Medicago* natural accession a high level of phenotypic diversity exists among populations.

We can therefore conclude that the two previous parameters NC and NS don't influence on the diversity between *M. ciliaris* population groups. Contrary to Graziano *et al.* (2010)whom reported the variability in pod characters like height, width and number of coils were large among Sicilian populations of *M. truncatula*.

The presence of homogenous between P4, P5 and P6 populations can be explicated by their geographical origin. They are prospected in different edges of the Oran great Sebkha. But the can be belonged by a single population at the beginning of their evolution and they are scattered by moving cattle along the meadows near Sebkha edges. The range of variability seemed to be discontinuous over this prospected salty area. Therecent study of Jabri *et al.* (2016) revealed considerable variation among the studied populations of *M. ciliaris*. L for the majority of recorded traits and the within-population variation was less significant.

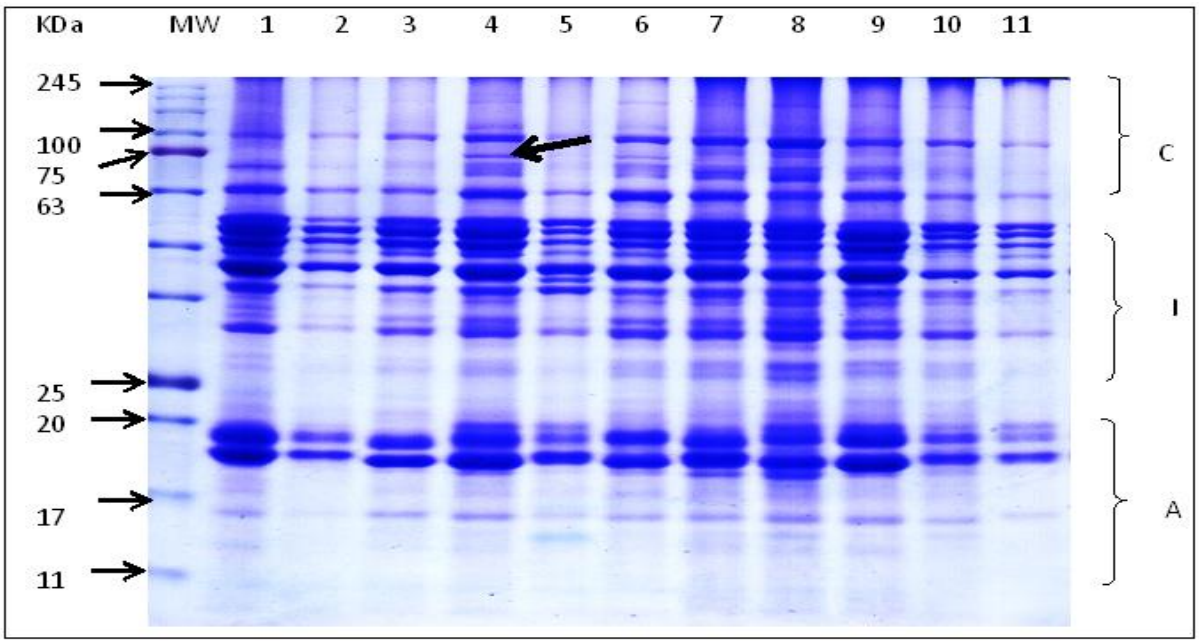

Fig. 3. The SDS-PAGE of globulins with 13.5 % acrylamide gel from mature seeds of 11 *M.ciliaris*. MW: molecular weight; 1, 2...11; corresponded population P1, P2....P11 respectively.

According to the mean values (μ) for all morphological traits indicate that the NEpop pop has a dwarfish aspect with comparison to FApop and Ref pop. The phenotypic variation coefficients are higher than the coefficients of genotypic variation for all characters analyzed, but the small deviation of the difference between these two coefficients indicates that these characters are very little influenced by the environment as indicated by several authors in Pearl millet (Lakshmana *et al.*, 2009; Béninga *et al.*, 2010). Comparing the average values of the parameters indicates that there is a slight reduction to the pop NE population, compared to those of the pop FA and pop Rf. This lowest value observed in the pop NE population could be related to its environmental conditions (salty area near Oran Great Sebkha).In addition High genetic coefficient (C*vg*) and phenotypic coefficient of variation (C*vp*) for pod weight, number of seeds per pod and the (ratio: diameter on high pod) suggested that the effectiveness of selection of these morphological traits. This result is parallel to the study of Badri *et al.* (2008), who founded low differentiation at quantitative traits in four *M. ciliaris* populations. Accordingly, Gitzendanner and Soltis (2000) were shown that geographical range was being a good predictor of the levels of genetic variation in plant.

Diameter and height were highly correlated with pod weight 0.83 and 0.68 respectively. This result is in line with that of Graziano *et al.* (2010) and Jabri *et al.* (2009) whom reported that pod size was correlated positive and significantly with pod weight. The pod size has played an important role in the seed dispersal for example *Medicago lupulina* has small indehiscent pods that facilitate long-distance seed dispersal by biotic and abiotic agents, whereas *M. ruthenica* has dehiscent pods and lacks effective mechanisms for seed dispersal (Yan *et al.*,2009).

Similarly positive and significant correlation between the following traits; pod weight, number of coils per pod, pod height, pod total length, number of spines per pod, number of seeds per pod, D/H ratio and pod diameter was also found. Strong association ($r=0.68$, $p<0.0001$) was observed between pod diameter and number of seeds per pod. It may be an advantage to have more seedlings in environments where the risk of seedling death is high such as Sebkha edges.

Because morphological traits only represent a part of the genetic variability, and because the organization of this diversity is highly subject to natural selection and environmental factors, knowledge of the evolution genetic of populations requires analysis of

neutral markers such as seed storage proteins. Neutral markers should help to specify the genetic diversity of populations (Salhi Hannachi *et al*., 1998). Comparative analysis of seed storage protein profiles has been a powerful for species identification and characterization, for clarifying taxonomic and evolutionary problems, and for studying genetic diversity (Ladizinsky and Hymowitz, 1979). Genetic diversity of seed proteins has been investigated in natural population's species of annual *Medicago* (Fyad-Lameche, 1998), in *medicago sativa* (Krochkoand Bewley, 2000) and in the model legume *M. truncatula* (Le Signor, 2005) using one-dimensional electrophoresis. In general, genetic variation in seed protein profiles has an important role in identification of varieties (Tripathy *et al*., 2015).

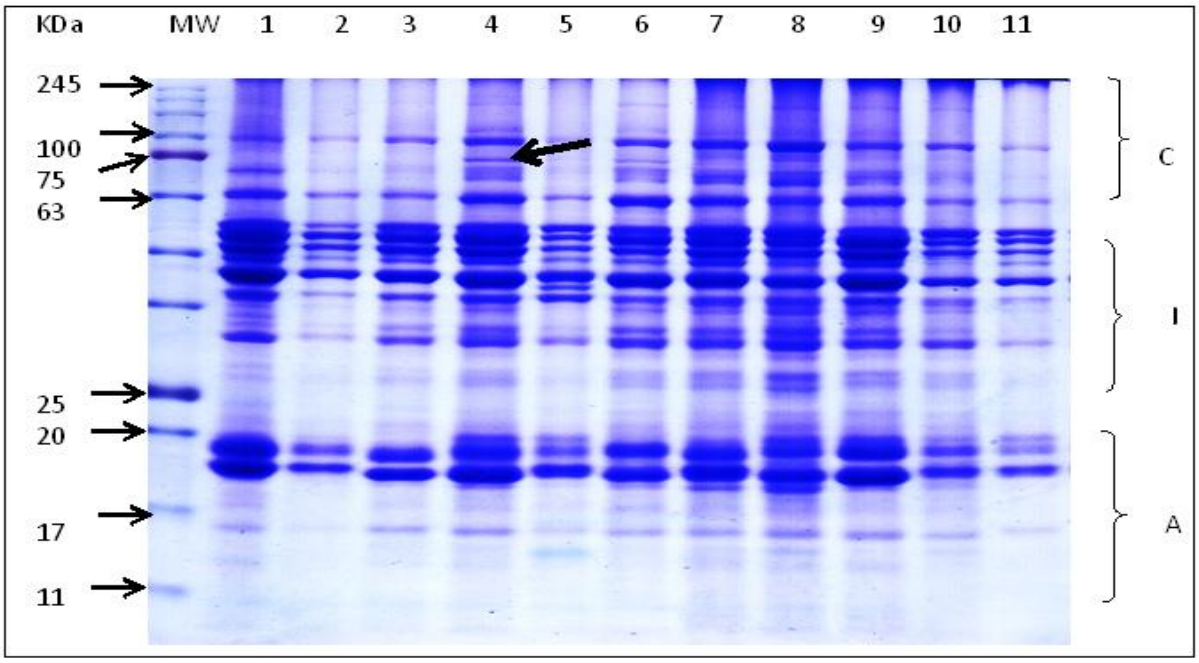

Fig. 3. The SDS-PAGE of globulins with 13.5 % acrylamide gel from mature seeds of 11 *M. ciliaris*. MW: molecular weight; 1, 2...11; corresponded population P1, P2....P11 respectively.

This method is a useful tool for this purpose. The results are in agreement with findings of Krochko and Bewley (2000) in *Medicago sativa*. The results are inparallel to the previous study with mini-gel (Moulai and Fyd-Lameche, 2014.These authors reported that each zone after migration contained major and minor bands with small variation in number and intensity.

According the software Gel analyser 10a, the total number of total storage protein bands detected in NEpo group, FApop group and Refpop groups is approximately 34, 45 and 36, respectively. The overall profiles of the total protein were similar in all population groups using 10% acrylamide gel, but the specific polypeptide band is resolved only in FA pop and Ref pop with molecular mass ~68 kDa and it is missing in NEpop. The results obtained by 13.5% acrylamide gel for globulins reveal also a common general aspect of the electrophoretic profiles among different populations with one specific band ~80 kDa that is specific only for NE pop group. These results are consistent with those obtained by mini gels in the same species (Moulaiand Fyd-Lameche, 2014). Previously, Faraghi *et al*. (2007) studied the variability of storage proteins 18 *M. sativa* perennial *alfalfa* genotypes based on SDS-PAGE. They reported a total of 61 bands, of which 16 are globulins. Habibi *et al* (2012), working on 18 genotype *M. sativa* L; they detected 24 bands. Recently, other authors are studding 13 accessions of *Medicago*; they showed that total proteins produced profiles with variation in bands for molecular weight (97 to 20 kDa) and in number (11 to 20 bands per profile). Each species are characterized by specific bands. But for the globulin fraction,

the profiles were relatively simple (3 to 6 band) of a certain species and more complex (9 to 12bands) for the rest of the species (Fyad-Lameche *et al*., 2016). Finally, genetic variability based on SDS-PAGE markers can play a key role in programs for selection of genotypes of interest.

In general, the results of this study show a small change to the total proteins and globulins in the same *M. ciliaris* populations. In contrast, significant variations were observed in pod morphology.

Conclusion

On the basis of the biometric results, the11*M.ciliaris*populations differing in their morphological pods texture was selected also for SDS-PAGE analysis to check the presence of seed storage protein diversity. Upon the genetic comparison, the NE pop population group appeared as differentiate population than the other population groups by their dwarfish morphological pod aspects and the presence or absence of specific polypeptide bands in denaturing condition. Using this information, we have been able to identify *M. ciliaris* suitable genotypes as parents for the generation of segregating populations useful for the genetic determination of legume seed traits that tolerate to salt stress. To use this promising species to valorize and enhance resources in future. Further studies on *M. ciliaris* are needed to clarify the genetic inter-population variability, considering a higher number of prospected populations and possibly extending to North Algeria populations.

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