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Characterization and Agronomic Evaluation of Local Populations of *Medicago minima* (L.) Collected in Algerian Steppe Area

[1]A. Chebouti, [2]A. Bekki, [3]M. Mefti and [4]N. Meziani
[1]National Institute of Agronomic Research of Algeria, Research Division Plant Genetic Resources, Algiers, Algeria
[2]Faculty of Sciences, University of Oran, Oran, Algeria
[3]Department of Crop Sciences, National School of Agronomy of El Harrach, Algiers, Algeria
[4]Faculty of Sciences, University of Boumerdes, Boumerdes, Agleria

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Corresponding Author:
A. Chebouti
National Institute of Agronomic Research of Algeria,
Research Division Plant Genetic Resources, Algiers, Algeria

ABSTRACT
M. minima (L.) Bart. is a pasture legume native to the Mediterranean Basin and is present throughout the Algerian territory. The work reported in this study was conducted in the aim to evaluate the diversity of pheno-morphological and agronomic traits in some local populations of *Medicago minima* coming from a 2004 survey conducted by INRA Algeria throughout the Djelfa area. Two field experiments were conducted during two cropping seasons 2009/2010 and 2010/2011 at the experimental farm of the Research Center of Plant Science of Baraki (INRAA). Analysis of variance and Principal Components Analysis (PCA) were performed on the complete set of the data. The results obtained show high genetic differentiation among populations of *M. minima* for all of the recorded traits. Population from Oued Touil region was the earliest and has presented the best vegetative development in winter and in spring. On the other hand, population from Charef presented the best pod and grain yields. The PCA based on morphological and agronomic data clearly discriminated the *M. minima* populations. For morphological characterization, the two principal components (PC1 and PC2) explained 84.85% of the total variation and for agronomic evaluation, they describe 79.79%. The large variability observed in all traits studied gives the opportunity to select a suitable plant material to exploit to regenerate degraded rangeland and crop-livestock farming systems in Algeria.

Key words: *Medicago minima*, genetic resources, characterization, evaluation

INTRODUCTION

Annual Legumes are a large group whose main center of taxonomic diversity is in the eastern Mediterranean and which displays a considerable variety of vegetative and reproductive morphologies (Ehrman and Cocks, 1996). Among crops reliable to promote pastoral zones that produce forage and restore destroyed pasture land especially in arid and semi-arid areas, the genus *Medicago* L. (Fabaceae) constitutes an important genetic resource (Haddioui *et al*., 2012). *Medicago* L. (Leguminosae), a predominantly Mediterranean genus, comprises a high number of species, annual herbs, herbaceous perennials and rare shrubs, many of which are markedly polymorphic (Brundu *et al*., 2004).

Annual medics are of great importance in Mediterranean pastures, as well as in South-western Australian and South American rangelands (Brundu *et al*., 2004). Medics are for the most part restricted to Mediterranean climates with winter rain

and long, hot, dry, summers. Annual medics are grown in medium rainfall zones (250-500 mm annual rainfall) (Bagheri *et al*., 2010). Annual *Medicago* are excellent candidates for use in sustainable agriculture systems, such as; pastures and cover crops (Dorry, 2010). Their high levels of hard seededness makes them well adapted to ley farming systems and to persistence in regions of unreliable rainfall (Nichols *et al*., 2007). Species of annual *Medicago* express high levels of N-fixation and protein production per hectare (Huguet *et al*., 1994). Zhu *et al*. (1998) reported that medics have the ability to fix 100-200 kg N per ha per year with appropriate rhizobia inocula. They have the potential to increase forage production and extend the grazing season (Interrante *et al*., 2011).

Among annuals medics, *Medicago minima* (L.) Bart. is a pasture legume native to the Mediterranean Basin and adjacent temperate regions and is one of the most widely distributed annual medics in both its area of origin and other parts of the world with semiarid temperate climate (Fresnillo, 2011). *M. minima* (L.) is high quality forage species, during spring in both overgrazed and properly managed areas (Busso *et al*., 1998). One other feature of *M. minima* with agronomic importance is, its adaptability to harsh climates. In Algeria, *M. minima* is present throughout the territory (Negre, 1959) and frequently present in arid and semi-arid grasslands, suggesting that populations adapted to local environment conditions could be identified and integrated into forage breeding programs.

The annual species of *Medicago* can play an important role in livestock feed. Unfortunately, the *M. minima* species is not exploited in our country. In order to identify genotypes, knowledge of the genetic diversity within and among natural populations of this species is necessary. The aim of the present work is to assess the variation in morpho-phenological and agronomic traits among local populations of *M. minima* collected from different regions of Djelfa and analyzed the relationships between these traits.

MATERIALS AND METHODS

Plant material and experimental site: Five local populations of *Medicago minima* were evaluated for their phenological and agronomic characters. These populations were collected in different sites of Djelfa area by the National Institute of Agronomic Research of Algeria (INRAA) in 2008. The origin sites of these populations are: Population from Ain Oussera (MmAO) (35°17'N; 2°57'E), population from Deldoul (MmDel) (34°15'N; 3°19'E), population from M'liliha (MmMli) (34°49'N; 3°49'E), population from Charef (MmCh) (34°40'N; 2°43'E) and population from Oued Touil (MmOT) (35°16'N; 2°33'E) (Fig. 1). The latitude and longitude of each site were taken using a portable Global Positioning System (GPS) receiver.

The trials were conducted at the experimental farm of the Research Center of Plant Science of Baraki, Algiers (INRAA). This area is characterized by a sub-humid climate with mild winters and hot summers. Mean annual rainfall varies between 600 and 900 mm, during the autumn-winter period. The mean minimum and maximum temperatures are 13.6 and 23.9°C, respectively.

Morphological characterization: The study was conducted during the 2009/2010 cropping season in glass greenhouse. Scarified seeds were sown on 23 December, 2009 in pots filled with 2/3 soil and 1/2 loam, at a rate of five seeds per pot and the plants were grown under uniform conditions. At emergence, we left two plants per pot. Pots were arranged in a completely randomized design with five replications. At flowering stage, the following traits were measured; appearance of the first flower (1F) (days from plant emergence to first flower), length of the branch that brought the first flower (LB1F) (cm), number of internodes of the branch that brought the first flower (NIN) and Number of Primary Branches (NPB). For pods and seeds, studied traits were Pod Diameter (DP) (mm), Thickness of Pod (TP) (mm), Number of Coils per Pod (NCP), Number of Seeds per Pod (NS/P), Length Seed (LS) (mm), Width Seed (WS) (mm). These measurements were taken for 25 pods and 20 seeds per population using a digital display caliper.

Agronomic evaluation: The experimentation was conducted during the 2010/2011 growing season. Populations were sown on 29 December, 2010 at a density of 100 seeds, previously scarified, per line 1 m long. Lines are spaced by 1.5 m. A randomized block design with 3 replications was used. Trial was conducted under rain-fed conditions. Weeds were manually removed. For each population, the following traits were measured during the development cycle of the plant: winter and spring vegetative development of plant. Width Winter (WW), Height Winter (HW), Width Spring (WS) and Height Spring (HS) (cm), date of Start Flowering (SF) (days from plant emergence to first flower per plant), End of Flowering (EF) (days from plant emergence to no flowers on line), date of Pod Formation Start (PFS) (days from plant emergence to first pod per plant) and date of Full Pod Formation (FPF) (time when there is maximum pods on line). At maturity, Total Number of Pods (TNP) per line, weight of pods per line in grams (PW), Total Number of Seeds (TNS) and seed weight per line in grams (SW) were also evaluated for each population.

Statistical analysis: Analysis of variance (ANOVA) was performed on the complete set of the data using, the GenStat software. Comparisons between means were made using, Least Significant Difference (LSD) at 5% probability level. Principal Components Analysis (PCA) was also performed to

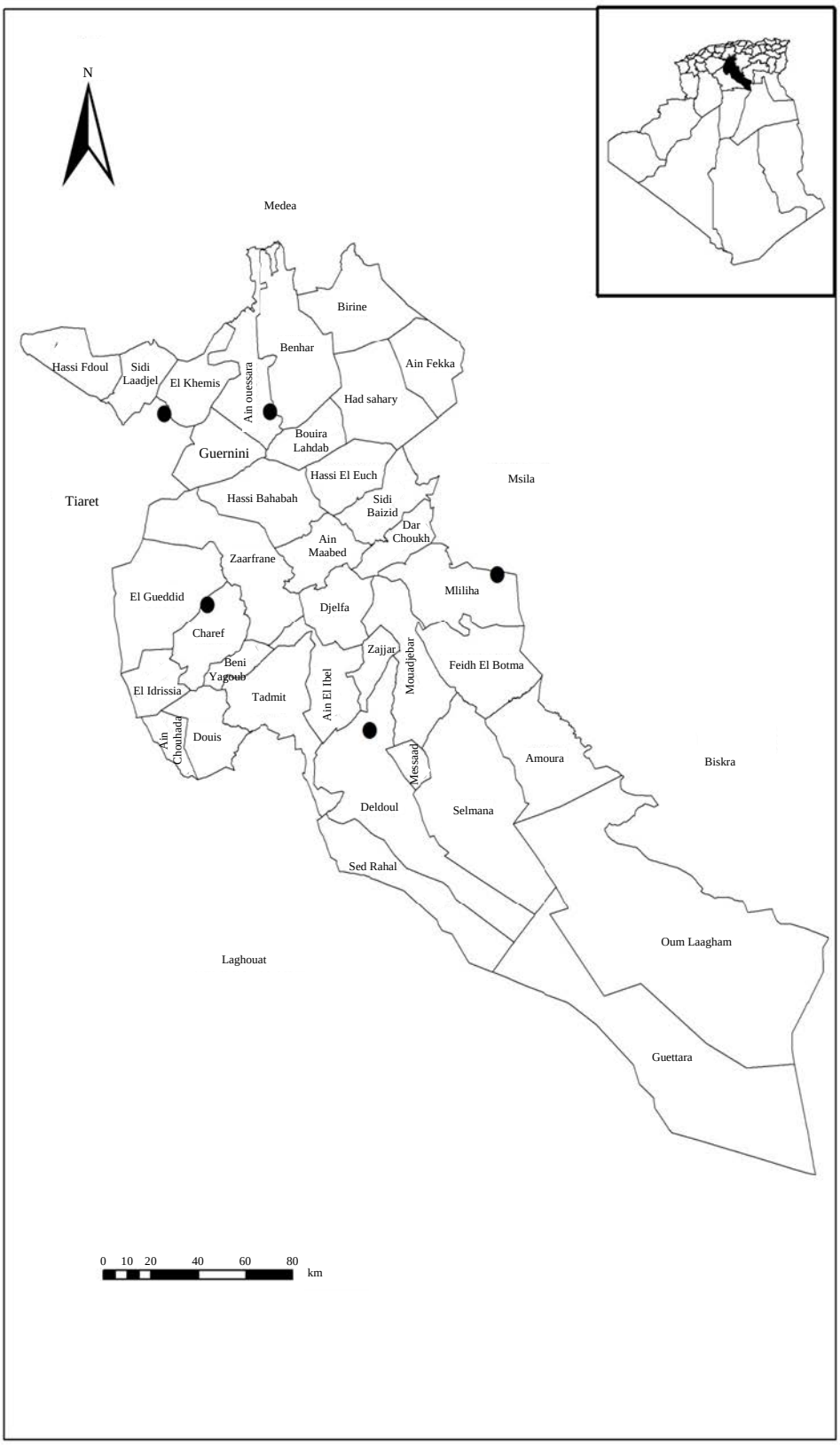

Fig. 1: Geographic locations of the collection sites of *Medicago minima* populations

establish the importance of different traits in explaining multivariate polymorphisms using Statistica 6.0 software. The relationships among the traits studied were tested using Pearson correlation coefficients.

RESULTS

Morphological characterization: Means and the range of variation of morphological traits are given in Table 1. We noted a high genetic variability in M. minima populations. The ANOVA shows highly significant differences between populations for all studied variables. For traits related to pods and seeds, the largest pods and high number of seeds per pod were recorded for the Mliliha population (DP = 4.52; TP = 3.93; NS/P = 5.44, respectively), while the population from Ain Oussera presented the largest seeds with LS = 2.44 and WS = 1.42. In contrast, the populations from Oued Touil had the smallest pods and seeds and the lowest number of seeds per pod. For traits related to plant, population from Mliliha presented the longest branch that brought the first flower (16.14 cm) and the highest number of internodes of this branch and of primary branches (6.91 and 6.58, respectively), whereas population from Oued Touil recorded the lowest values.

Several correlations were observed between the various studied traits (Table 2). Seed length was positively correlated with seed width. Thus, populations with a high number of seeds per pod presented thick pods and a high number of coils per pod. Pod diameter was highly and positively correlated with thickness of the pod. Populations that have the longest branch presented a high number of coils per pod and a large seeds. Moreover, number of primary branches was positively correlated with diameter and thickness of pod. No relationships were observed between owering time and morphological traits.

The PCA, performed on morphological traits and including the altitude of populations sites origin, revealed significant differentiation between populations (Fig. 2). The two principal components (PC1 and PC2) plots describe 84.85% of the total variation. The PC1 explained 64.27% of the variation and was negatively related with the majority of variables. The PC2 absorbs 20.58% of the variation and was positively correlated with flowering time and negatively with altitude. The PC1 puts in evidence two groups of populations. The group, represented by populations from Ain Oussera, Mliliha and Deldoul regions, is distinguished from Oued Touil population by the large pods and seeds, the high number of seeds per pod and the good plant development. Populations from Charef region was plotted on the negative side of PC2 and characterized by early flowering and it seems to come from higher altitude sites.

Agronomic evaluation: The ANOVA performed on the populations of *M. minima* showed significant differences for all of the studied characteristics (Table 3). The spring vegetative development of all populations was significantly, higher than winter development. The populations seem to grow better in spring than in winter. There were large

Table 1: Means value of the morphological traits measured in *Medicago minima* populations

Populations	LS	WS	TP	DP	TNP	NS/P	1F	LB1F	NIN	NPB
MmAO	2.44^{a}	1.42^{a}	3.55^{b}	4.14^{b}	3.52^{a}	5.32^{a}	102.33^{a}	16.99^{a}	6.33^{b}	6.33^{b}
MmCh	2.23^{b}	1.14^{c}	3.32^{c}	4.15^{b}	3.12^{b}	5.04^{a}	99.16^{b}	14.06^{b}	5.67^{b}	5.42^{c}
MmOT	1.92^{c}	1.07^{d}	2.99^{d}	3.89^{c}	3.00^{b}	4.56^{b}	102.50^{a}	13.73^{b}	5.92^{b}	5.33^{c}
MmMli	2.25^{b}	1.24^{b}	3.92^{a}	4.52^{a}	3.44^{a}	5.44^{a}	102.16^{a}	16.14^{a}	6.92^{a}	7.25^{a}
MmDel	2.32^{b}	1.25^{b}	3.56^{b}	4.24^{b}	3.52^{a}	5.44^{a}	102.42^{a}	15.25^{b}	5.92^{b}	6.58^{b}
G.Mean	2.23	1.24	3.47	4.19	3.32	5.16	101.71	15.23	6.5	6.18
p-value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
LSD	0.1203	0.0751	0.1878	0.189	0.2358	0.372	0.5395	1.669	0.5184	0.5983

G.M: General mean, LSD: Least significant difference, 1F: Appearance of the first flower, LB1F: Length of the branch that brought the first flower, NIN: Number of internodes of the branch that brought the first flower, NPB: Number of primary branches, DP: Diameter of pod, TP: Thickness of the pod, NC/P: Number of coils per pod, NS/P: Number of seeds per pod, LS: Length of seed, WS: Width of seed

Table 2: Correlation coefficients among morphological traits recorded for *Medicago minima* populations

Variables	LS	WS	TP	DP	TNP	NS/P	1F	LB1F	NIN	NPR
LS	1									
WS	0.891*	1								
TP	0.689	0.608	1							
DP	0.529	0.374	0.961**	1						
NTP	0.86	0.861	0.808	0.641	1					
NS/P	0.87	0.726	0.914*	0.829	0.928*	1				
1F	-0.04	0.308	0.159	0.011	0.414	0.12	1			
LB1F	0.806	0.948*	0.768	0.567	0.882*	0.772	0.421	1		
NIN	0.278	0.46	0.781	0.728	0.53	0.514	0.478	0.715	1	
NPR	0.564	0.578	0.943*	0.881*	0.84	0.856	0.461	0.766	0.829	1

Significant at p<0.05, p<0.01, p<0.001. 1F: Appearance of the first flower, LB1F: Length of the branch that brought the first flower, NIN: Number of internodes of the branch that brought the first flower, NPB: Number of primary branches, DP: Diameter of pod, TP: Thickness of the pod, NC/P: Number of coils per pod, NS/P: Number of seeds per pod, LS: Length of seed, WS: Width of seed

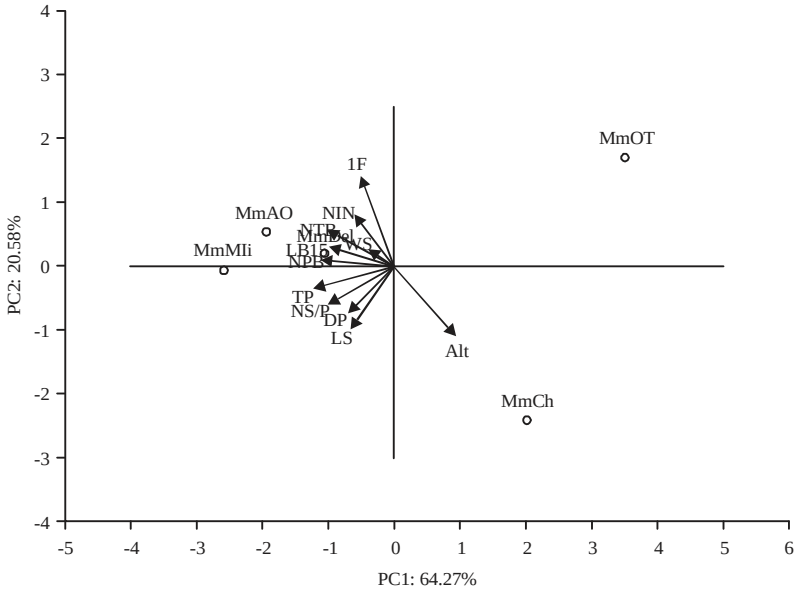

Fig. 2: Plot of the two first principal components of *Medicago minima* morphological traits, 1F: Appearance of the first flower, LB1F: Length of the branch that brought the first flower, NIN: Number of internodes of the branch that brought the first flower, NPB: Number of primary branches, DP: Diameter of pod, TP: Thickness of the pod, NCP: Number of coils per pod, NS/P: Number of seeds per pod, LS: Length of seed, WS: Width of seed

Table 3: Means value of the phonological and agronomic traits measured in *Medicago minima* populations

Populations	WW	WS	HW	HS	SF	EF	PFS	FPF	TNP	WP	TNS	SW
MmAO	10.80^{bc}	36.90^{c}	3.13^{b}	8.20^{b}	81.00^{b}	119.00^{b}	85.00^{b}	112.00^{b}	7552.00^{bc}	251.00^{a}	40177.00^{bc}	68.3^{b}
MmCh	8.60^{c}	34.10^{c}	3.20^{b}	9.23^{b}	84.00^{a}	120.00^{b}	88.00^{a}	116.00^{a}	15867.00^{a}	248.00^{a}	79968.00^{a}	101.6^{a}
MmDel	8.53^{c}	26.00^{d}	3.17^{b}	6.73^{c}	84.00^{a}	119.00^{b}	88.00^{a}	112.00^{b}	5536.00^{c}	104.00^{b}	30116.00^{c}	49.2^{c}
MmMli	11.60^{b}	37.90^{bc}	3.17^{b}	9.03^{b}	82.00^{b}	125.00^{a}	85.00^{b}	114.00^{ab}	12206.00^{ab}	269.00^{a}	66399.00^{ab}	89.8^{ab}
MmOT	17.20^{a}	81.20^{a}	5.30^{a}	15.67^{a}	74.00^{c}	116.00^{c}	77.00^{d}	113.00^{ab}	11296.00^{b}	286.00^{a}	51510.00^{bc}	59.2^{bc}
G. Mean	11.35	43.24	3.59	9.77	81.00	90.70	84.60	113.60	10491.00	231.00	53634.00	73.6
p-value	0.001	0.001	0.005	0.001	0.001	0.001	0.001	0.066	0.003	0.032	0.005	0.034
Lsd	2.882	5.80	1.049	1.49	1.46	1.945	2.019	3.075	4149.10	110.70	21889.20	33.33

GM: General mean, LSD: Least significant difference, WW: Width winter, HW: Height winter, WS: Width spring, HS: Height spring, SF: Date of flowering start, EF: End of flowering, PFS: Date of pod formation start, FPF: Date of full pod formation, TNP: Total number of pods, WP: Weight of pods, TNS: Total number of seeds, SW: Seed weight

differences between populations of *M. minima* for width and height of plant. For development plant, in winter the means value of WW and HW were 11.35 and 3.59 cm, respectively, whereas in spring, the means value of WS and HS were 43.24 and 9.77 cm. Oued Touil Population distinguished from others by a better vegetative development in winter and spring. On average, dates of the flowering start, end of flowering and pod formation start were 81, 91 and 85 days after emergence, respectively. Population from Oued Touil region was earliest for the flowering traits (SF and EF) and for pod formation compared to other populations. Pod and seed yield of *M. minima* populations were highly variable. Mean value of pod and seed-related traits were highly variable. The mean values of total number of pods, weight of pods, seed number and seed weight were 10491 pods, 231 g, 53634 seeds and 73.6 g, respectively. From results obtained, population from Charef region produced a higher number of pods and seeds per line than the other populations.

The correlation coefficients are given in Table 4. Vegetative development traits are highly and positively, correlated between them. Thus, populations, which presented a good winter and spring vegetative development were those presenting the earliest flowering and pod formation dates. Flowering date was very highly and positively correlated with pod formation date. For traits related to pod and seed production, total number and weight of pods are positively correlated with total number and weight of seeds. We also noted that, populations produced high seed number presented a high seed weight. No significant correlations were observed between flowering time and traits related to pod and seed yield. Moreover, full pod formation was positively, related to total number of pods and total number and weight of seeds.

The principal component analysis was performed to visualize the main sources of variability between populations. The PCA based on both phenological and agronomic data clearly discriminated the populations in *M. minima*.

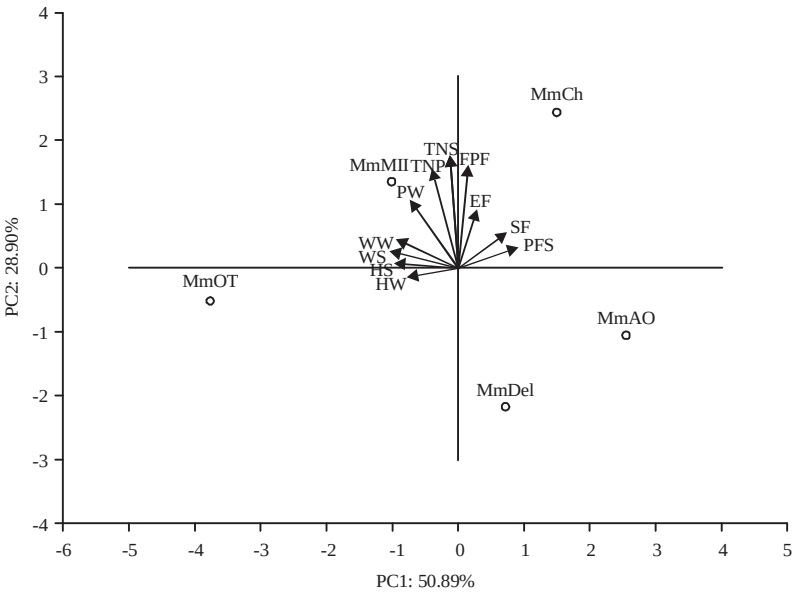

Fig. 3: Plot of the two first principal components of *Medicago minima* phonological and agronomic traits, WW: Width winter, HW: Height winter, WS: Width spring, HS: Height spring, SF: Date of flowering start, EF: End of flowering, PFS: Date of pod formation start, FPF: Date of full pod formation, TNP: Total number of pods, PW: Weight of pods, TNS: Total number of seeds, SW: Seed weight

Table 4: Correlation coefficients among phenological and agronomic traits recorded for *Medicago minima* populations

Variables	WW	WS	HW	HS	SF	EF	PFS	FPF	TNP	PW	TNS	SW
WW	1.00											
WS	0.91	1.00										
HW	0.82	0.85	1.00									
HS	0.89	0.98	0.87	1.00								
SF	-0.91	-0.96	-0.81	-0.90	1.00							
EF	-0.37	-0.51	-0.51	-0.46	0.50	1.00						
PFS	-0.87	-0.86	-0.68	-0.85	0.86	0.17	1.00					
FPF	-0.10	0.02	-0.09	0.11	0.18	0.30	-0.07	1.00				
TNP	0.12	0.18	0.14	0.33	-0.07	0.24	-0.22	0.70	1.00			
PW	0.51	0.47	0.33	0.51	-0.48	0.08	-0.39	0.31	0.72	1.00		
TNS	0.01	0.04	0.00	0.19	0.06	0.38	-0.13	0.70	0.99	0.70	1.00	
SW	-0.17	-0.19	-0.20	-0.06	0.24	0.48	0.05	0.58	0.88	0.69	0.94	1.00

Value in bold are significant at p<0.05, p<0.01, p<0.001. WW: Width winter, HW: Height winter, WS: Width spring, HS: Height spring, SF: Date of flowering start, EF: End of flowering, PFS: Date of pod formation start, FPF: Date of full pod formation, TNP: Total number of pods, PW: Weight of pods, TNS: Total number of seeds, SW: Seed weight

The PCA identified two principal components, which together accounted for 79.79% of the total variance (Fig. 3). The PC1 explained 50.89% of the variation and was positively influenced by flowering and pod formation start and negatively by vegetative growth traits and yield seed. The PC2 which explained 28.9% of the variation is principally, correlated positively with total pod number, pod weight, total number seed and end flowering. In fact, population from Oued Touil region, projected on the negative side of the PC1, was distinguished from Ain Oussera population by good winter and spring vegetative growth and early flowering and pod formation. Populations from Charef and Mliliha were plotted on the positive side of PC2 and presented high pod number, high pod weight, high seed number and late end flowering. In contrast, population from Deldoul region was the earliest and characterized by low pod and seed yield.

DISCUSSION

Populations of *M. minima* showed highly significant inter-population differences for all of the recorded morpho-phenological and agronomic traits. The variability between populations collected in different regions of Djelfa was large. Several morphological investigations on *M. truncatula* populations of Morocco showed great vegetative development diversity (Haddioui *et al*., 2012; El Hansali *et al*., 2007). The variability in pod and seed characters among *M. minima* populations was large. Similar results were observed by Bullita *et al*. (1994) and Graziano *et al*. (2010) in Sardinian populations of *Medicago plymorpha*. Species of the genus *Medicago* draw their importance from the large variability that they present (Chaulet, 1995). Such variability could be useful, when selecting for an increase in

self-reseeding capacity (Graziano *et al*., 2010). In fact, in the present study, pod size was positively related to the number of seeds per pod. Cheima *et al*. (2012) report the significant correlations between pod weight and pod height, pod diameter and seed weight and between pod and seed weight in *M. ciliaris*. According to Bullita *et al*. (1994), the variation for pod size indicates that several populations could be useful in a selection program to enhance self reseeding because small pod size helps self reseeding. In addition, the spring vegetative development of all populations was significantly higher than what was observed in winter. Similar results were reported by Prosperi *et al*. (1993), indicated that the natural populations of *Medicago sative* are characterized by good spring growth. Fresnillo (2011) reports that dry matter production in spring represented more than 60% of the total aboveground production of the herbaceous layer in a year in *M. minima*.

Flowering time differentiation observed in *M. minima* populations can be related to their original environmental conditions, particularly temperature. Del Pozo and Aronson, (2000) indicated that annual legumes show both ecotypic differentiation and a high degree of plasticity in flowering time. Flowering date varied between 74 and 84 days, while pod formation date ranged from 77-88 days. Del Pozo *et al*. (2001) reports that onset of flowering ranged from 128-138 and from 78-124 days in populations of burr medic collected in Sardinia and Chile, respectively. Early-flowering accessions tended to present a good vegetative development in both winter and spring. Arraouadi *et al*. (2006) mention negative correlations between flowering and some traits related to vegetative growth in *M. truncatula*. Lodge *et al*. (1993) have also found negative correlations between winter production (dry mater) and days of flowering.

Populations of *M. minima* evaluated in this study were shown to produce high quantity of pods and seeds. In fact, number and weight of pods and seeds are closely related, thus seed yield depend to pods number. This indicates the ability of these populations for regenerating degraded rangeland in arid and semi-arid environments. According to Abdelkefi and Marrakchi (2000), annual species of *Medicago* produce a great quantity of indehiscent pod containing hard seeds and thus, corroborate our finding. Thus, most of the evaluated medics were adequate for the establishment of a viable seed bank that would allow regeneration at the start of subsequent pasture phases (Dorry, 2010). Traits related to pod and seed production were very highly correlated. This relationship suggests that these traits vary in similar ways. These results are in accordance with those of Cocks (1988), who indicted that seed yield was closely related to number of pods in all ecotypes of annual medics (*Medicago* spp.). High seed yield potential is of particular importance in medics (annual *Medicago* species) which self-regenerate year after year from the seeds left buried in the soil (Somaroo, 1988).

CONCLUSION

In conclusion, this study has shown the existence of a large variability in all the traits examined among local populations of *M. minima*. This variability offers possibilities for selection of plant material adapted to our environmental conditions. Several correlations were observed between the various studied parameters. We noted strong relationship between earlier owering and vegetative development traits. From the obtained results, population from Oued Touil region was the earliest for flowering and has presented the best vegetative development capacity in both winter and spring. Furthermore, population from Charef region was the most interesting population with regard to it high pods and seeds production.

On the other hand, studied populations of *M. minima* presented a good vegetative development in spring than in winter and produced also appreciable seed and pod yield could be recommended for use in forage breeding programs in order to improve forage production and regenerate degraded rangeland in arid and semi-arid environments in Algeria. Further research should be carried in order to develop local populations of *M. minima* for resistance to difficult cropping conditions (especially, water stress) and atmospheric nitrogen fixing potential.

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