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Research Article

Variation in Morphological Traits and Yield Evaluation among Natural Populations of *Medicago truncatula* and *Medicago laciniata*

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Abstract

Background and Objective: Legumes of the genus *Medicago* have particular ecological and agro-pastoral importance in the Mediterranean regions. In order to develop local species by identifying and selecting cultivars adapted, populations of *Medicago truncatula* and *Medicago laciniata* were evaluated at the experimental farm of INRAA (Baraki) in 2015/2016 cropping season. **Materials and Methods:** Ten populations of *M. truncatula* and *M. laciniata* from different regions of Djelfa were used. The study is based on pheno-morphological and agronomic traits. ANOVA and principal components analysis (PCA) were performed on the complete set of the data. **Results:** ANOVA showed significant differences between populations for the majority of studied parameters. Also, the two principal components (PC1 and PC2) explained 72.73% of the total variation for morphological variation and 79.43% for agronomic evaluation trial. **Conclusion:** Large variability among natural populations of *M. truncatula* and *M. laciniata* was observed for all studied traits. This variability can be exploited in fodder breeding programs to select an adapted plant material for arid and semi-arid area.

Key words: *M. truncatula*, *Medicago laciniata*, genetic diversity, evaluation, seed yield

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Algeria is one of the richest areas of genetic diversity, where a wide variety of agro-ecological environments can be identified, nevertheless the random nature of annual rainfall and unpredictable and severe droughts often aggravate the situation of Algerian agriculture, which has a huge forage deficit, where animals are often subjected to periods of frequent food shortages¹.

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 resource². *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 polymorphic³. With *Trifolium* genus, *Medicago* represent the most important genres in Algeria. Their number would be more⁴ than 50.

Legumes of the genus *Medicago* are of special ecological and agro-pastoral importance in the Mediterranean regions. They include forage species expressing high levels of N-fixation and protein production per hectare⁵. Medics grown as regenerating pasture in the agro-pastoral Mediterranean systems or cereal farming systems are an important feed resource not only as green forage throughout the growing season but also as stubbles and pods in summer and early autumn⁶. Other benefits can be listed for incorporating annual *Medicago* into farming systems. These include their potential to increase forage production and extend the grazing season⁷, ability to increase soil fertility and structure and capacity to break disease and pest life cycles of crops when grown in rotation⁸, as well as their high levels of hard seededness makes them well adapted to ley farming systems and to persistence in regions of unreliable rainfall⁹.

M. truncatula and *M. laciniata* have two different geographical distributions through the world and in Algeria. This distribution is controlled by both edaphic and climatic conditions. The *M. truncatula* present a considerable

variability genetic, resulting in many differences in phenotype and growth characteristics between ecotypes¹⁰. Key attributes of *M. truncatula* include its self fertile mature, its prolific seed production and its rapid generation time¹¹. Unlike the majority of legumes, *M. truncatula* is well accessible to molecular tools and genetic analyzes. It is therefore, adapted to the study the biological mechanisms of the major functions specific to legumes¹². The *M. laciniata* is probably native to southern Mediterranean countries and the eastern Mediterranean, where it appears to be adapted to steppe soils and deserts¹³. This species shows exceptional persistence under difficult conditions¹⁴ and can be particularly useful for improving dryland rangeland or used in rotation with barley in these areas to increase productivity and stability of systems¹⁵.

Huge possibilities exist to develop annual medics crop through the identification and selection of adapted cultivars belonging to several species. For that, the present study analyzed the morphological variation and assessed the pod and seed production in 10 natural populations of *Medicago truncatula* and *Medicago laciniata*.

MATERIALS AND METHODS

Germplasm collection and experimental site: Ten natural populations of *Medicago truncatula* and *Medicago laciniata* collected in different sites of Djelfa area by the National Institute of Agronomic Research of Algeria (INRAA) in 2008 were used. The latitude and longitude of each site were taken using a portable Global Positioning System (GPS) receiver (Table 1).

The experiments were conducted during the 2015/2016 cropping season at the experimental farm of the Research Center of Plant Science of Baraki, Algiers (INRAA). This area is characterized by a subhumid 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.

Table 1: List of *M. laciniata* and *M. truncatula* populations and original collecting sites

Species	Géographical origin	Altitude (m)	Latitude	Longitude
<i>Medicago laciniata</i>	Bouiret Lahdab (MIBL)	830	35°12'66"	3°02'88"
	Deldoul (MIDel)	768	34°15'73"	3°19'76"
	Messaad (MIMes)	950	34°15'78"	3°20'12"
	Mliliha (MIMli)	806	34°48'89"	3°48'94"
	Oued Touil (MmOT)	718	35°16'43"	2°33'23"
<i>Medicago truncatula</i>	Bouiret Lahdab (MtBL)	830	35°12'66"	3°02'88"
	Oued Touil (MtOT)	718	35°16'43"	2°33'23"
	Mliliha (MtMli)	806	34°48'89"	3°48'94"
	Ain Oussera (MtAO)	758	35°17'08"	2°57'37"
	Charef (MtCh)	960	34°40'31"	2°43'13"

Morphological variation: For each population of both species, the following pod and seed traits were measured on plant material from the collection mission: Pod diameter (DP, mm), pod thickness of pod (TP, mm), number of coils of the pod (NCP), number of seeds per pod (NSP), length of seed (LS, mm) and width of seed (WS, mm). These measurements were taken for 25 pods and 20 seeds per population using a digital display caliper.

Then the scarified seeds of each populations were sown on 31 December, 2015 in pots filled with 2/3 soil and 1/2 loam at a rate of five seeds per pot in glasshouse. The plants were grown under uniform conditions. At emergence, it left two plants per pot. Pots were arranged in a completely randomized design with five replications. At flowering stage, the following traits were measured in 10 plants per population: Appearance of the first flower (1F) (days between plant emergence and appearance of the 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).

Yield evaluation: For this experimentation, populations were sown on 15 December, 2015 at a density of 100 seeds, previously scarified, per line of 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. For each population, the following traits were measured: Flowering time (FT) (days between plant emergence and appearance of one flower per plant), pod formation time (PFT) (days between plant emergence appearance of one pod per plant), total number of pods per line (TNP), pods weight per line (PW, g), total number of seeds (TNS) and seed weight per line (SW, g).

Statistical analysis: Data were subjected to one-way analysis of variance (ANOVA) using the GenStat software edition 12 and the measured traits means were compared between populations with Least Significant Difference (Lsd) test at 5% probability level. Principal components analysis (PCA) was performed to establish the importance of different traits in explaining multivariate polymorphisms, using Statistica 6.0 software. The relationships among measured traits were tested using Pearson correlation coefficients.

RESULTS

Morphological variation

Morphological variability: Analysis of variance showed significant differences between populations for whole studied traits, except for number of internodes, number of primary branches for *M. truncatula*, where differences are not significant (Table 2). For *M. truncatula*, the mean values of seed length, seed width, pod diameter and pod thickness were 2.77, 1.79, 5.21 and 5.57 mm, respectively and they were 2.53 and 1.36 mm, 4.54 and 4.84 mm for *M. laciniata*. For both species, population from Bouiret Lahdab was distinguished by the largest pods and seeds, as well as a high number of seed per pod. For plant development traits, *M. truncatula* has presented the longest branch (16.00 cm), while *M. laciniata* recorded the highest number of internodes and of primary branches (6.30 and 6.57, respectively).

Relationships among traits: Several correlations among morphological traits values were noted in both species (Table 3, 4). High and positives correlations were found

Table 2: Means value of the morphological traits measured in *M. truncatula* and *M. laciniata* populations

Popul.	LS	WS	TP	DP	NCP	NSP	1F	LB1F	NIN	NPB
MtBL	3.54 ^a	2.07 ^a	6.93 ^a	6.98 ^a	5.56 ^a	7.16 ^a	95 ^c	17.09 ^a	5.83	5.75
MtOT	3.24 ^b	1.85 ^b	5.29 ^b	6.18 ^b	4.56 ^b	6.12 ^b	95 ^c	17.73 ^a	6.66	5.91
MtMli	3.13 ^b	1.76 ^c	4.60 ^c	5.61 ^c	4.28 ^c	5.56 ^c	98 ^a	13.22 ^c	6.16	6.00
MtAO	2.92 ^c	1.58 ^d	5.3 ^b	3.80 ^e	3.28 ^d	4.60 ^d	97 ^b	16.38 ^a	6.50	6.33
MtCh	2.99 ^c	1.70 ^c	3.92 ^d	5.29 ^d	3.00 ^e	4.36 ^d	96 ^b	15.59 ^{ab}	6.25	6.41
G.M.	2.77	1.79	5.21	5.57	4.13	5.56	96	16.0	6.28	6.08
Lsd	0.129	0.072	0.345	0.256	0.242	0.427	0.857	1.937	0.596	0.548
p-value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.068	0.089
MIBL	2.86 ^a	1.49 ^a	6.09 ^a	5.25 ^a	5.36 ^a	8.08 ^a	102 ^b	14.45 ^a	6.58 ^a	6.42 ^b
MIDel	2.62 ^b	1.41 ^b	4.41 ^b	4.86 ^b	3.68 ^b	5.68 ^b	103 ^a	13.17 ^b	5.50 ^b	6.08 ^b
MIOT	2.52 ^{bc}	1.34 ^c	4.37 ^b	4.67 ^b	3.44 ^b	5.88 ^b	102 ^b	15.77 ^a	6.92 ^a	7.75 ^a
MIMes	2.23 ^d	1.25 ^d	3.97 ^c	4.76 ^b	3.56 ^b	5.00 ^b	102 ^b	14.89 ^a	6.75 ^a	6.67 ^b
MIMli	2.44 ^c	1.29 ^{cd}	3.84 ^c	4.67 ^b	3.4 ^b	5.84 ^b	102 ^b	13.72 ^b	5.75 ^b	5.92 ^b
G.M.	2.53	1.36	4.54	4.84	3.88	6.09	102	14.40	6.30	6.57
Lsd	0.116	0.069	0.242	0.220	0.277	0.449	0.374	1.477	0.637	0.894
p-value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

G.M: General mean, Lsd: Least significant difference, LS: Length of seed, WS: Width of seed, DP: Diameter of pod, TP: Thickness of the pod, NCP: Number of coils of the pod, NSP: Number of seeds per pod, 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

Table 3: Relationship between morphological traits in *M. truncatula* populations

Variables	LS	WS	TP	DP	NCP	NSP	1F	LB1F	NIN	NPB
LS	1									
WS	0.990***	1								
TP	0.790	0.723	1							
DP	0.918*	0.953**	0.491	1						
NCP	0.966**	0.926*	0.814	0.843	1					
NSP	0.975**	0.941**	0.564	0.857	0.996***	1				
1F	-0.667	-0.707	-0.595	-0.646	-0.539	-0.608	1			
LB1F	0.343	0.349	0.545	0.233	0.279	0.353	-0.871*	1		
NIN	-0.589	-0.603	-0.412	-0.519	-0.483	-0.457	0.109	0.267	1	
NPB	-0.926*	-0.883*	-0.737	-0.834	-0.986***	-0.979**	0.485	-0.238	0.375	1

Significant levels, *p<0.05, **p<0.01 and ***p<0.001

Table 4: Relationship between morphological traits in *M. laciniata* populations

Variables	LS	WS	TP	DP	NCP	NSP	1F	LB1F	NIN	NPB
LS	1									
WS	0.975*	1								
TP	0.878*	0.891*	1							
DP	0.800	0.845	0.950*	1						
NCP	0.796	0.811	0.970**	0.982**	1					
NSP	0.901*	0.844	0.943*	0.872	0.928*	1				
1F	0.087	0.172	-0.275	-0.197	-0.351	-0.329	1			
LB1F	-0.201	-0.24	0.055	-0.16	-0.033	-0.010	-0.677	1		
NIN	-0.118	-0.124	0.244	0.085	0.200	0.136	-0.781	0.951*	1	
NPB	-0.111	-0.127	0.012	-0.240	-0.151	-0.086	-0.357	0.924*	0.810	1

Significant levels, *p<0.05, **p<0.01 and ***p<0.001

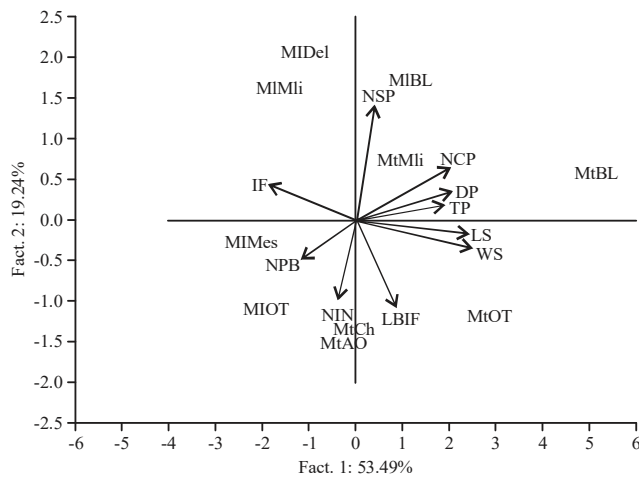


Fig. 1: PCA1 performed on morphological traits for the 10 studied populations of *M. truncatula* and *M. laciniata*

DP: Diameter of pod, TP: Thickness of the pod, NCP: Number of coils of the pod, NSP: Number of seeds per pod, LS: Length of seed, WS: Width of seed, 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

between pods and seeds traits, this suggest that these measured characters vary in similar ways. Therefore, populations that showed the largest pods have the greatest

seeds. In *M. laciniata*, number of internodes and number of primary branches were positively correlated with the longest branch that brought the first flower. On the other hand, in *M. truncatula*, number of primary branches is negatively correlated with LS, WS, NCP and NSP (Table 3).

Pattern of morphological variation: The principal components analysis identified two principal components (PCA1), which together accounted for 72.73% of the total variance (Fig. 1). The first axis explained 53.49% of the variation and was positively influenced by traits related to pods and seeds and negatively by days between plant emergence and appearance of the first flower and number of primary branches. The second axis which explained 19.24% of the variation was correlated positively with number of seeds per pod and negatively with length of the branch that brought the first flower and number of internodes. PCA1 differentiate clearly the populations of the two species. In fact, populations of *M. truncatula* from Bouiret Lahdab, Oued Touil and Mliliha, plotted on the positive side of axis 1 were characterized by largest pods and greatest seeds, contrary to populations from Messaad and Oued Touil of *M. laciniata*. While populations from Charef and Ain Oussera of *M. truncatula*, distributed on the negative side of axis 2 have presented the longest branch and the higher number of internodes than those of *M. laciniata* from Bouiret Lahdab, Mliliha and Deldoul.

Table 5: Means value of the phenological and yield traits measured in *M. truncatula* and *M. laciniata* populations

Popul.	DF	DFG	NTG	PTG	NTgr	RdtGr
MtAO	73	78	9894 ^a	418 ^{ab}	45512 ^a	133.8 ^a
MtBL	75	82	8103 ^{ab}	340 ^{bc}	45054 ^a	121.6 ^{ab}
MtCh	74	80	8063 ^b	488 ^a	35156 ^{ab}	95.6 ^{bc}
MtMli	74	82	4321 ^c	260 ^c	25928 ^b	72.9 ^c
MtOT	70	77	4727 ^c	289 ^c	28927 ^b	81.9 ^c
G.Mean	73	80	7022	359	36116	101.2
Lsd	4.125	4.238	1811.2	95.8	10504	29.03
p-value	ns	ns	0.001	0.003	0.007	0.006
MIBL	81 ^b	81 ^b	5200 ^c	84.2 ^b	31200 ^d	64 ^{cd}
MIDel	84 ^a	88 ^a	7348 ^d	207.1 ^a	41739 ^b	78.2 ^{bc}
MIMes	82 ^b	86 ^a	3567 ^e	82.6 ^b	20972 ^c	54.5 ^d
MIMli	81 ^b	82 ^b	9457 ^b	206.9 ^a	55231 ^a	93.9 ^{ab}
MIOt	74 ^c	77 ^c	11443 ^a	233.4 ^a	57215 ^a	107.7 ^a
G.Mean	80	83	7403	162.9	41271	79.7
Lsd	1.454	2.119	1573	34.28	9076.2	17.17
p-value	0.001	0.001	0.001	0.001	0.001	0.001

G.M: General mean, LSD: Least significant difference, FT: Flowering time, PFT: Pod formation time, TNP: Total number of pods per line, PW: Pods weight per line, TNS: Total number of seeds, SW: Seed weight per line

Table 6: Relationships among phenological and yield traits in *M. truncatula* populations

Variables	FT	PFT	TNP	PW	TNS	SW
FT	1					
PFT	0.366	1				
TNP	0.368	-0.081	1			
PW	0.309	-0.239	0.826***	1		
TNS	0.397	-0.013	0.925***	0.666**	1	
SW	0.338	-0.075	0.932***	0.655**	0.991***	1

Significant levels, * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$

Table 7: Relationships among phenological and yield traits in *M. laciniata* populations

Variables	FT	PFT	TNP	PW	TNS	SW
FT	1					
PFT	0.885***	1				
TNP	-0.658**	-0.581*	1			
PW	-0.394	-0.241	0.922***	1		
TNS	-0.542	-0.515	0.982***	0.921***	1	
SW	-0.662**	-0.581*	0.985***	0.904***	0.970***	1

Significant levels, * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$

Yield evaluation

Yield traits variability: For flowering and pod formation times, the variance analysis showed highly significant differences between population of *M. laciniata*, but no significant differences were noted between populations of *M. truncatula* (Table 5). For both species, the population from Oued Touil has been the earliest population. Flowering time varied from 70-75 calendar days for *M. truncatula* and from 74-84 days for *M. laciniata*. The tested populations of *M. truncatula* and *M. laciniata* differed significantly for pod and seed production. The highest pod and seed numbers were obtained by *M. laciniata* (7403 and 41271, respectively), while the highest weights of pods and seeds were observed in *M. truncatula* (395 and 101.2 g, respectively). From results obtained, the highest yield was recorded by Ain Oussera population in *M. truncatula* (133.8 g) and by Oued Touil population in *M. laciniata* (107.7 g).

Relationships among yield traits: In both species, high and positives correlations were found between traits related to pods and seeds production (Table 6, 7). On the other hand, the earliest populations have produced high number of pod and seed ($r = -0.658^{**}$ and $r = -0.542^{*}$, respectively) in *M. laciniata*, but no relationships were observed between flowering and pod formation times and yield component in *M. truncatula*.

Pattern of agronomic variation: The two first components of PCA2 explained 79.43% of the total variance (Fig. 2). The first component accounted for 48.96% of the total variation and was positively correlated with pods weight and seeds weight and negatively with flowering and pod formation time. The second component explained 30.47% of the total variation and was dominated by the total number of pods and total number of seeds. Figure 2 shows the distribution of the *M. truncatula* and *M. laciniata* populations. Population of

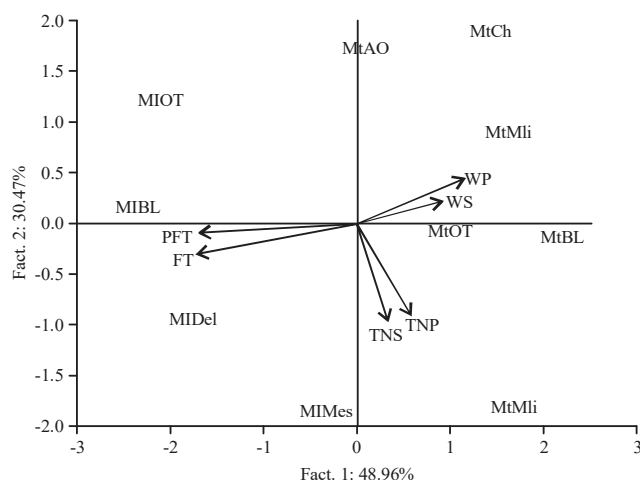


Fig. 2: PCA2 performed on yield traits for the 10 studied populations of *M. truncatula* and *M. laciniata*
 FT: Flowering time, PFT: Pod formation time, TNP: Total number of pods per line, PW: Pods weight per line, TNS: Total number of seeds, SW: Seed weight per line

M. truncatula from Bouiret Lahdab, Oued Touil and Mliliha, projected on the positive side of the first axis were distinguished from by high yields of pod and seed and were the earliest populations. Although, populations of *M. laciniata* from Messaad and Mliliha, plotted on the negative side of the second axis, produced high number of pods and seeds, contrary to the populations of *M. truncatula* coming from Ain Oussera and Charef areas.

DISCUSSION

Significant variation for all traits measured found in *M. truncatula* and *M. laciniata* suggested that a large genetic variability exists. Such variability can be exploited in different breeding program of forage species to select adapted material. High genetic differentiation was noted by Badri *et al.*¹⁶ among natural Tunisian populations of *M. laciniata* and by El Hansali *et al.*¹⁷ in Morocco populations of *M. truncatula*. Our results are in accordance with those noted by Chebouti *et al.*¹⁸, Chebouti *et al.*¹⁹ and Chebouti *et al.*²⁰ in Algerian local populations of *M. minima* and *M. truncatula*. In the present study, *M. truncatula* has the largest seeds and pods than *M. laciniata*. According to Graziano *et al.*²¹, it could be advantageous in dry environments to have large seeds. In fact, pod and seed traits were closely related between them in both species.

Flowering time can be related to original environmental conditions of populations, particularly temperature¹⁹.

Flowering times vary significantly between species. Obtained results showed that *M. truncatula* species is earlier than *M. laciniata*. On average, the flowering time of *M. truncatula* was 73 and 80 days for *M. laciniata*. According to Del Pozo and Aronson²², annual legumes show both ecotypic differentiation and a high degree of plasticity in flowering time. Graziano *et al.*²¹ reported that the different pattern of phenological differentiation observed in the Sardinian, Sicilian and Chilean populations can be explained by the different range of bioclimatic and edaphic conditions within each environment. Sulas *et al.*²³ mentioned that flowering of *Trifolium subteranum* and annual *Medicago* occurs in response to day length and temperature, but cultivars vary in their response to these factors considerably. Fayd-Lamache²⁴ indicated that flowering period is a useful index when choosing cultivars for particular rainfall zones.

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 soil²⁵. In this study, pod and seed production varied widely among populations of *M. laciniata* and *M. truncatula*. However, *M. laciniata* produced a high number of pods and seeds, in contrast *M. truncatula* had the highest yield of pods and seeds. For regenerating degraded rangeland in arid and semi-arid areas, it could be an advantage to produce a lot of seeds. According to Abdelkefi and Marrakchi²⁶, annual species of *Medicago* produce a great quantity of indehiscent pod containing hard seeds. In fact, seed yield was closely depended to pod and seed numbers suggested that these traits vary in similar ways. In many legumes, one of the effective factors on seed yield is pod number and commonly there is a linear function between pod number and seed yield²⁷. Cocks and Ehemann²⁸ mentioned that in annual medics, seed yield was depended to pods number. However, it have noted that early flowering populations of *M. laciniata* have presented higher pod and seed yields. On the contrast, Graziano *et al.*²¹ reported that seed yield were higher in later flowering populations of *M. polymorpha*.

CONCLUSION

We noted significant variation within populations of *M. truncatula* and *M. laciniata* for all the traits examined. This variation can be exploited in fodder breeding programs to select an adapted plant material in order to exploit in pastures in Algeria. From the obtained results, Ain Oussera population of *M. truncatula* and Oued Touil population of *M. laciniata* were the most interesting populations with regard to their high pods and seeds production. The both

populations can play an important role in improving forage quality and regenerating degraded rangeland in arid and semi-arid environments. Further study is needed to characterise symbiotic rhizobia with the aim to identify strains able to nodulate annual medics.

SIGNIFICANCE STATEMENT

Populations of *M. truncatula* and *M. laciniata* exhibited a large variability for most studied traits. This variability would be of a great to develop an adapted material to exploit in pastures and crop-livestock farming systems in Algeria. Moreover, Ain Oussera population of *M. truncatula* and Oued Touil population of *M. laciniata* were the most productive populations in pods and seeds and can recommended for arid and semi-arid environments.

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