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Morpho-phenological diversity among natural populations of *Medicago polymorpha* of different Tunisian ecological areas

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Medicago polymorpha **is a herbaceous legume that can be a useful pasture plant, in particular, in regions with a Mediterranean climate. The genetic variation in 120 lines of *M. polymorpha* sampled from five regions in Tunisia was characterized on the basis of 16 morpho-phenological characters. Results from analysis of variance (ANOVA) showed that differences among populations and lines existed for all traits, with population explaining the greatest variation for measured traits. The populations of Enfidha and Soliman were the earliest flowering, while those of El Kef, Bulla Regia and Mateur were the latest. El Kef and Mateur exhibited the highest aerial dry weight while the lowest value was found for Soliman. Moderate to lower levels of heritability (H^2) were registered for investigated traits. There was no significant association between pairwise population differentiation (Q_{ST}) and geographical distances. Studied lines were clustered into three groups with 59 for the first group, 34 for the second group, and 27 lines for the third group. The lines of the first two groups showed the largest length of stems while those of the second group had the highest number of leaves. The variation of quantitative traits among populations was influenced by the altitude, temperature and relative humidity. Overall, the high levels of within population variation and the lack of correlation between population differentiation and geographical distances suggest a potentially important rate of long-distance seed dispersal and confirm the role played by natural selection in the population structure of Tunisian populations of *M. polymorpha*.**

Key words: *Medicago polymorpha*, populations, quantitative traits, population differentiation, environmental parameters.

INTRODUCTION

The genus *Medicago,* with about 87 species of herbs and shrubs widespread from the Mediterranean to central Asia (Small and Jomphe, 1989; Small, 2010), includes the widely cultivated major forage crop *Medicago*

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sativa (alfalfa, lucerne) and the legume model species *Medicago truncatula*. In Tunisia, the genus *Medicago* is well represented and it is an extremely rich and diverse gene pool (Seklani et al., 1996). *Burr Medic (Medicago polymorpha*) is an annual herbaceous legume, diploid (2n = 14) and self-compatible species. *M. polymorpha* can be a useful pasture plant, in particular, in regions with a Mediterranean climate (Salhi Hannachi et al., 1998). It had aroused great interest due to high nutritious quality, highly palatability and N-fixing plan in neutral soil (Loi et al., 1993, 1995). *M. polymorpha* is a species of Mediterranean origin, but its species range is wide spread throughout the world. The wide diffusion and adaptability can be explained by its low sensitivity to photoperiod and vernalization (Aitken, 1981). Heyn (1963) identified three botanical varieties of this species: *brevispina* with spineless or tubercled pods; *polymorpha*, spined and *vulgaris* spined but with pods smaller than *polymorph*a. In Tunisia, *M. polymorpha* grows in a range of environments from humid to upper arid stages (Abdelkefi et al., 1996).

In general, plant growth and reproduction is dependent on the effects of genotype and the abiotic and biotic factors. Sufficient genetic diversity is very important for plants to survive in changing climate conditions. Traditionally, plants diversity is assessed by morphological descriptors. Morpho-phenological characterization of natural populations of *M. polymorpha* may provide valuable insights into the traits and underlying genetics needed for meeting challenges of the future environmental conditions. Morphological characters are generally quantitative, having a mono- or polygenic determinism. Understanding of morphological characters facilitates the identification of desirable traits and their genetic determinants (Tar'an et al., 2005; Arraouadi et al., 2011; Badri et al., 2011). Sixteen quantitative traits considered as descriptors for *M. truncatula*, *Medicago laciniata* and *Medicago ciliaris* populations (Badri et al., 2007, 2010; Arraouadi et al., 2009; Lazrek et al., 2009) were measured for the lines of *M. polymorpha*. Genetic diversity may appear spatially structured at different scales, such as among neighboring individuals, subpopulation, and population (Escudero et al., 2003).

Knowledge of spatial genetic structures provides a valuable tool for inferring the evolutionary forces such as selective pressures and drift (De Kort et al., 2012). Low gene flow due to spatial isolation of populations may even increase the degree of local differentiation (Hendry, 2002). Nevertheless, phenotypic plasticity rather than genetic differentiation may be an alternative way of matching genotypes to environment; indeed increasing environmental variation favors higher levels of plasticity (Hahn et al., 2012).

The current study aims to: (i) analyze the morpho-phenological diversity within and among natural populations of *M. polymorpha*, and (ii) assess the relationship between phenotypic variation among populations and the site-of-origin environmental parameters. In this study plant performance was studied under greenhouse conditions, where plants grew in common environments for multiple generations. Thus, population and within population effects should be due to genetics rather than due to maternal environmental effects.

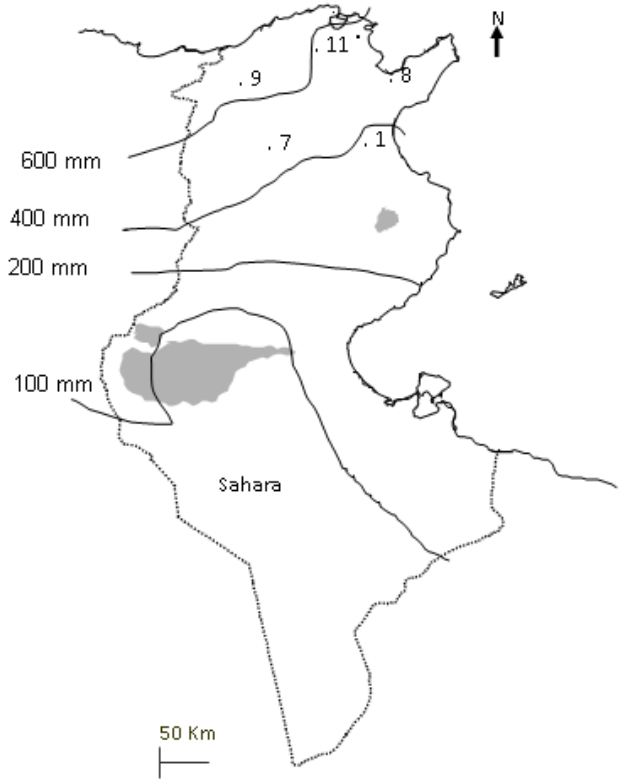

Figure 1. Map of Tunisia with the location of natural populations of *Medicago polymorpha* from which studied lines were collected. Enfidha (1), El Kef (7), Soliman (8), Bulla Regia (9), and Mateur (11).

MATERIALS AND METHODS

Plant material

Five populations of *M. polymorpha* collected from different eco-geographical regions (Enfidha, El Kef, Soliman, Bulla Regia, and Mateur) (Figure 1) in Tunisia were used. These populations were sampled in July, 2009 and represented three different bioclimatic stages, ranging from sub-humid to lower semi-arid environments (Table 1). At each location, 24 pods were collected randomly from each population. The neighboring samples were four meters apart to avoid sampling the same genotype more than once. Distances between populations were at least 43 km inbred lines. To minimize maternal environmental effects on trait expression, inbred lines of *M. polymorpha* were developed by single-seed descent at the F3 generation under greenhouse conditions.

Table 1. Environmental factors of the collection sites of Tunisian populations of *Medicago polymorpha.*

Population (code)	Number of studied lines	Longitude (E)	Latitude (N)	Bioclimatic stage	Altitude (m)	Texture	Electro-conductivity (mmho/cm)	Mean annual rainfall (mm)	Mean temperature (°C)	Mean relative Humidity (%)
Enfidha (TNP1)	24	10°220'	36°070'	Lower semi-arid	2	Clay	0.6	350	20.40	68.33
El Kef (TNP7)	24	35°831'	94°255'	Upper semi-arid	500	Sandy loam	0.8	450	17.60	61.00
Soliman (TNP8)	24	10°320'	36°410'	Upper semi-arid	2	Loamy sand	1.4	600	19.20	77.00
Bulla Regia (TNP9)	24	25°844'	37°429'	Sub-humid	200	Sandy loam	0.8	600	19.17	62.67
Mateur (TNP11)	24	09°400'	37°010'	Sub-humid	37	Loamy sand	3.2	600	18.9	60.8

Morpho-phenological characterization

The experiment was conducted under greenhouse conditions at the Centre of Biotechnology of Borj Cedria (CBBC) in spring, 2014. Twenty four lines per population were used. Seeds were scarified using sandpaper and were transferred in pots (diameter = 17 cm; deep = 13 cm) of two liters filled with soil of the CBBC and compost of sphagnum (2:1). Six replicates per line were used, giving a total of 720 plants, which were organized into a randomized complete three blocks design. Each plant was grown in an individual pot in greenhouse with a mean temperature of 25°C. Plants were grown in well-irrigated treatment (100% of field capacity). Three replicates of each genotype from each block were harvested at the formation of the first green pod and three at the end of plants' lifecycle. Sixteen quantitative traits were measured for the lines of M. *polymorpha*. Eleven of these were related to vegetative growth: days from emergence to first true leaf (D1L, days), days from emergence to sixth leaf (D6L, days), length of stems (LS, cm), length of roots (LR, cm), number of ramifications (NR), number of leaves (NL), aerial fresh weight (AFW, g), aerial dry weight (ADW, g), root dry weight (RDW, g), root dry weight and aerial dry weight ratio (RDW/ADW), aerial dry weight at second harvest (ADWh2, g). The remaining characters were related to flowering time and pods production: days from emergence to first flower (FLOR, days), number of pods (NPOD), weight of pods (WPOD, g), weight of 100 pods (W100P, g), and harvest index (HI). The aerial and root dry weights was estimated after drying in an oven at 70°C for 48 h.

Statistical analyses

To test for population and line nested within population effects on the 14 traits measured from greenhouse grown plants, ANOVAs were performed using general linear models (GLM) procedure (type III) in SPSS version 16 (2007 Rel 1600 SPSS Inc., Chicago, IL, USA) where population and lines were considered as fixed factors. Comparison of population means of measured traits was performed using the Duncan's multiple range test at 5%.

The estimation of variance among populations (Vp) and lines (Vg) was performed using the VARCOMP procedure in SPSS treating the population and lines as random factors, relying on Restricted Maximum Likelihood (REML) method. The residual variance between the replicates of the same genotype (=line) was considered as the environmental variance (Ve). Broad-sense heritability (H^2) of the traits was estimated as the ratio of the genetic variance on the sum of the genetic and environmental variances (Badri et al., 2007). Population differentiation (Q_{ST}) for quantitative traits was computed as reported for a predominantly autogamous species (Badri et al., 2015) as $Q_{ST}=Vp/(Vp+Vwp)$, where Vp is the variance among populations and Vwp the variance within populations. Pairwise Q_{ST} was only estimated for the traits showing significant variation among populations. Correlations between Q_{ST} and geographical distances were analyzed using the Mantel test (Mantel, 1967) in XLSTAT software v 7.5 (Addinsoft, USA). The matrix of geographical distance between populations was calculated by measuring the shortest distance between two points in the map, using geographical coordinates for each site.

Pearson correlations between measured traits were estimated using Correlate procedure in SPSS software. Clustering analysis of lines and populations was performed based on dissimilarity matrix using Euclidean distances estimated on the mean line and population values, respectively, with the Ward's method in XLSTAT software. A discriminant analysis (DA) was performed on means of measured traits for the groups of lines and populations. Cluster and discriminant analyses were performed on means of 7 and 12 traits showing significant differences for lines and populations, respectively.

Five environmental parameters of sampling sites of populations of *M. polymorpha* were examined: altitude (m), electro-conductivity (mmho/cm), mean annual rainfall (mm), temperature (°C) and relative humidity (%). Soil samples were collected from three points at each site and they were analyzed at the Laboratory of Soils at the Ministry of Agriculture, Tunisia. The temperature (T), the mean annual rainfall (R), and the relative humidity (H) were provided by the National Institute of Meteorology, Tunis, Tunisia. Pearson correlations between 12 traits, showing significant variation among populations, and environmental parameters were estimated using Correlate procedure in SPSS software. Significance level was set to 0.05 and adjusted for multiple comparisons by Bonferroni correction (Badri et al., 2007).

RESULTS

Morpho-phenological variation among populations

Results from the ANOVA showed that the variation of measured traits was explained by the effects of population and lines within population (Table 2). The largest effect was found for population. Of the 16 traits, 11 differed among populations and 7 differed among lines. The populations of El Kef, Bulla Regia and Mateur were the latest flowering while those of Enfidha

Table 2. Contribution of population line within population treatment population x treatment (P x T) and line x treatment (L x T) interaction effects on measured traits for populations of *Medicago polymorpha.*

		D1L	D6L	FLOR	LS	LR	NR	NL	AFW	ADW	RDW	RDW/ADW	ADWh2	NPOD	WPOD	W100P	HI
Population	F	6.07	1.44	23.02	1.82	2.02	3.11	4.05	3.46	17.80	3.57	0.71	21.12	5.05	3.29	2.96	1.62
	P	0.000	0.228	0.000	0.134	0.101	0.020	0.005	0.012	0.000	0.010	0.585	0.000	0.001	0.015	0.025	0.17
Line(Pop)	F	1.80	0.81	3.68	2.12	1.12	2.19	1.96	0.98	4.87	0.83	0.87	2.19	1.08	1.00	1.10	1.29
	P	0.005	0.829	0.000	0.001	0.305	0.000	0.002	0.536	0.000	0.793	0.738	0.000	0.371	0.507	0.345	0.130

Significant (P ≤ 0.05), non significant (P>0.05), F: coefficient of Snedecor-Fisher. Days from emergence to first true leaf (D1L days); days from emergence to sixth leaf (D6L days); days from emergence to first flower (FLOR days); length of stems (LS cm); length of roots (LR cm); number of ramifications (NR); number of leaves (NL); aerial fresh weight (AFW g); aerial dry weight (ADW g); root dry weight (RDW g); root dry weight and aerial dry weight ratio (RDW/ADW); aerial dry weight at second harvest (ADWh2 g); number of pods (NPOD); weight of pods (WPOD g); weight of 100 pods (W100P g); harvest index (HI).

Table 3. Mean values and coefficient of variation (CV), variance among populations (Vp), genetic variance (Vg), environmental variance (Ve), heritabilities (H^2) and population differentiation for quantitative traits (Q_{ST}) for measured traits for populations of *Medicago polymorpha.*

Parameter	TNP1	TNP7	TNP8	TNP9	TNP11	F	*P*	CV	Vp	Vg	Ve	H^2	Q_{ST}
D1L	9.75±1.73^{a}	7.99±7.48^{c}	9.31±1.95^{ab}	9.03±1.89^{bc}	9.06±1.52^{c}	6.07	0.000	2.18	0.23	0.06	13.77	0.00	0.02
D6L	26.13±4.54^{a}	25.50±3.01^{a}	25.34±3.39^{a}	25.56±3.00^{a}	25.08±3.68^{a}	1.44	0.228	0.74	0.00	0.00	12.74	0.00	0.00
FLOR	50.48±11.25^{b}	60.38±18.88^{a}	47.10±9.91^{b}	57.47±8.99^{a}	54.69±9.41^{a}	23.02	0.000	1.32	26.13	103.37	66.39	0.61	0.13
LS	32.06±8.56^{b}	38.61±13.01^{a}	31.41±17.70^{ab}	37.48±8.41^{a}	33.63±10.73^{ab}	1.82	0.134	2.22	7.71	37.83	116.93	0.24	0.05
LR	7.86±3.40^{ab}	9.10±3.76^{a}	7.26±3.05^{b}	10.06±14.10^{ab}	8.63±3.59^{ab}	2.02	0.101	5.19	0.18	14.44	37.29	0.28	0.00
NR	2.38±1.16^{ab}	3.81±8.75^{a}	2.03±1.04^{b}	2.66±1.29^{a}	2.33±1.10^{a}	3.11	0.020	8.90	0.19	0.74	15.32	0.05	0.01
NL	65.80±31.58^{bc}	70.60±34.40^{abc}	58.91±31.61^{c}	80.78±37.93^{a}	76.36±43.01^{ab}	4.05	0.005	3.19	49.33	277.66	1046.00	0.21	0.04
AFW	3.19±1.58^{ab}	3.97±2.59^{a}	2.30±1.21^{c}	3.81±1.90^{a}	3.12±1.73^{ab}	3.46	0.012	3.67	0.37	0.81	2.94	0.22	0.09
ADW	0.59±0.33^{bc}	1.10±0.78^{a}	0.42±0.26^{c}	0.73±0.35^{b}	0.79±1.09^{a}	17.8	0.000	5.71	0.06	0.10	0.34	0.23	0.12
RDW	0.04±0.04^{b}	0.15±0.37^{a}	0.93±5.74^{b}	0.04±0.03^{b}	0.06±0.07^{b}	3.57	0.010	66.53	0.01	0.02	5.90	0.00	0.00
RDW/ADW	0.07±0.07^{a}	0.13±0.33^{a}	1.60±9.37^{a}	0.08±0.09^{a}	0.09±0.09^{a}	0.71	0.585	74.42	0.03	0.02	16.67	0.00	0.00
ADWh2	1.05±0.87^{b}	1.63±1.17^{a}	0.87±0.44^{b}	1.11±0.58^{b}	1.05±1.95^{b}	21.12	0.000	46.23	0.00	0.00	264.01	0.00	0.00
NPOD	24.02±13.22^{a}	14.92±8.35^{b}	22.42±13.27^{ab}	24.77±14.99^{a}	24.47±13.40^{a}	5.05	0.001	3.49	14.48	6.17	168.38	0.04	0.08
WPOD	0.84±0.51^{a}	0.71±0.46^{ab}	0.71±0.46^{ab}	0.73±0.50^{ab}	0.67±0.39^{b}	3.29	0.015	28.50	0.00	0.04	25.15	0.00	0.00
W100P	3.70±1.59^{b}	4.53±1.90^{a}	3.42±2.56^{bc}	3.28±2.86^{bc}	2.99±1.18^{c}	2.96	0.025	67.46	0.00	0.00	15830.00	0.00	0.00
HI	0.98±0.64^{a}	0.79±0.94^{a}	1.92±0.68^{a}	0.75±0.63^{a}	0.79±0.59^{a}	1.62	0.17	23.22	0.21	0.87	16.92	0.05	0.01

Enfidha (TNP1), El Kef (TNP7), Soliman (TNP8), Bulla Regia (TNP9), and Mateur (TNP11). Days from emergence to first true leaf (D1L days); days from emergence to sixth leaf (D6L days); days from emergence to first flower (FLOR days); length of stems (LS cm); length of roots (LR cm); number of ramifications (NR); number of leaves (NL); aerial fresh weight (AFW g); aerial dry weight (ADW g); root dry weight (RDW g); root dry weight and aerial dry weight ratio (RDW/ADW); aerial dry weight at second harvest (ADWh2 g); number of pods (NPOD); weight of pods (WPOD g); weight of 100 pods (W100P g); harvest index (HI). Standard deviation (SD), means of each trait followed by the same letters are not significantly different between studied populations of *M. polymorpha.*

and Soliman were the earliest (Table 3). The highest aerial dry weight (ADW) was registered for El Kef population and Mateur while the lowest values were for Enfidha and Soliman. The largest weight of pods (WPOD) was observed for Enfidha, El Kef, Soliman and Bulla Regia while

Table 4. Correlations between pairwise Q_{ST} matrix and geographical distances (GD) and between measured traits for natural populations of *Medicago polymorpha*.

		D1L	D6L	FLOR	LS	LR	NR	NL	AFW	ADW	RDW	RDW/ADW	ADWh2	NPOD	WPOD	W100P	HI
GD	**r**	**-0.29**	**ND**	**0.85**	**ND**	**ND**	**0.76**	**0.64**	**0.69**	**0.48**	**-0.66**	**ND**	**0.92**	**0.16**	**ND**	**-0.22**	**0.88**
	P	**0.770**	**ND**	**0.325**	**ND**	**ND**	**0.357**	**0.473**	**0.415**	**0.604**	**0.459**	**ND**	**0.639**	**0.863**	**ND**	**0.844**	**0.323**
D1L		1.00															
D6L		0.44*	1.00														
FLOR		0.19*	0.17*	1.00													
LS		-0.07	0.06	0.48*	1.00												
LR		0.02	0.09	0.11	0.23*	1.00											
NR		-0.03	-0.04	0.27*	0.16*	0.03	1.00										
NL		-0.03	-0.10	0.39*	0.43*	0.11	0.05	1.00									
AFW		-0.14*	-0.01	0.43*	0.44*	0.08	0.27*	0.52*	1.00								
ADW		-0.05	0.00	0.56*	0.42*	0.11	0.24*	0.67*	0.45*	1.00							
RDW		-0.02	-0.01	-0.01	0.02	-0.02	0.00	0.07	0.02	-0.01	1.00						
RDW/ADW		-0.01	0.00	-0.01	0.02	-0.03	0.00	0.06	0.01	-0.02	1.00*	1.00					
ADWh2		-0.01	-0.06	0.01	-0.01	-0.04	0.01	0.05	-0.03	-0.02	-0.02	-0.04	1.00				
NPOD		0.05	0.00	-0.11	0.06	0.22*	0.12*	0.18*	0.11	0.09	0.03	0.03	0.09	1.00			
WPOD		0.03	0.22*	-0.01	-0.01	0.29*	0.03	0.05	0.05	-0.05	0.11	0.12	0.01	-0.01	1.00		
W100P		0.03	0.22*	0.01	-0.09	0.05	-0.16*	-0.16*	-0.14*	-0.15*	0.06	0.06	-0.08	-0.08	1.00*	1.00	
HI		0.05	0.11	-0.07	-0.24*	-0.04	-0.03	-0.26*	-0.31*	-0.35*	0.29*	0.44*	-0.13*	0.08	0.11	0.03	1.00

*Significant ($P \leq 0.05$), non significant ($P>0.05$). Non determinant (ND); Days from emergence to first true leaf (D1L days); days from emergence to sixth leaf (D6L days); days from emergence to first flower (FLOR days); length of stems (LS cm); length of roots (LR cm); number of ramifications (NR); number of leaves (NL); aerial fresh weight (AFW g); aerial dry weight (ADW g); root dry weight (RDW g); root dry weight and aerial dry weight ratio (RDW/ADW); aerial dry weight at second harvest (ADWh2 g); number of pods (NPOD); weight of pods (WPOD g); weight of 100 pods (W100P g); harvest index (HI).

the lowest value was for Mateur. Higher variation within populations (CV > 50%) was observed for root dry weight (RDW), the ratio RDW/ADW and weight of 100 pods (W100P), moderate levels (20% < CV < 50%) were registered for aerial dry weight at the second harvest (ADWh2), WPOD and HI and lower values (CV < 20%) were noted for the remaining characters.

Higher broad-sense heritability ($H^2 > 0.4$) was found for flowering time, moderate levels ($0.2 \leq H^2 \leq 0.4$) were noted for length of stems (LS), length of roots (LR), number of leaves (NL), aerial fresh weight (AFW), and ADW, and lower values ($H^2 < 0.2$) were for the remaining traits (Table 3). Moderate population differentiation (Q_{ST}) was registered for flowering time (FLOR) and ADW, and lower levels were for the remaining traits. There was no significant association ($P > 0.05$) between the pairwise Q_{ST} matrix and geographical distances as confirmed by a Mantel test (Table 4).

Among the 120 possible correlations between measured traits, 38 correlations were significant and 27 of them were positive (Table 4). Most of correlations between the parameters of aerial growth were positive. The flowering time was positively correlated with days from emergence to first true (D1L) and sixth (D6L) leaves and the LS, the number of ramifications (NR), NL, AFW, and ADW. The NPOD was positively correlated with LR, NR, and NL. The HI was negatively correlated with LS, NL, AFW, ADW, and ADWh2.

Cluster analysis

The hierarchical cluster analysis was used to examine the aggregation patterns of 120 lines of *M. polymorpha*. Studied lines were clustered into three groups (Figure 2). A first group contained 59

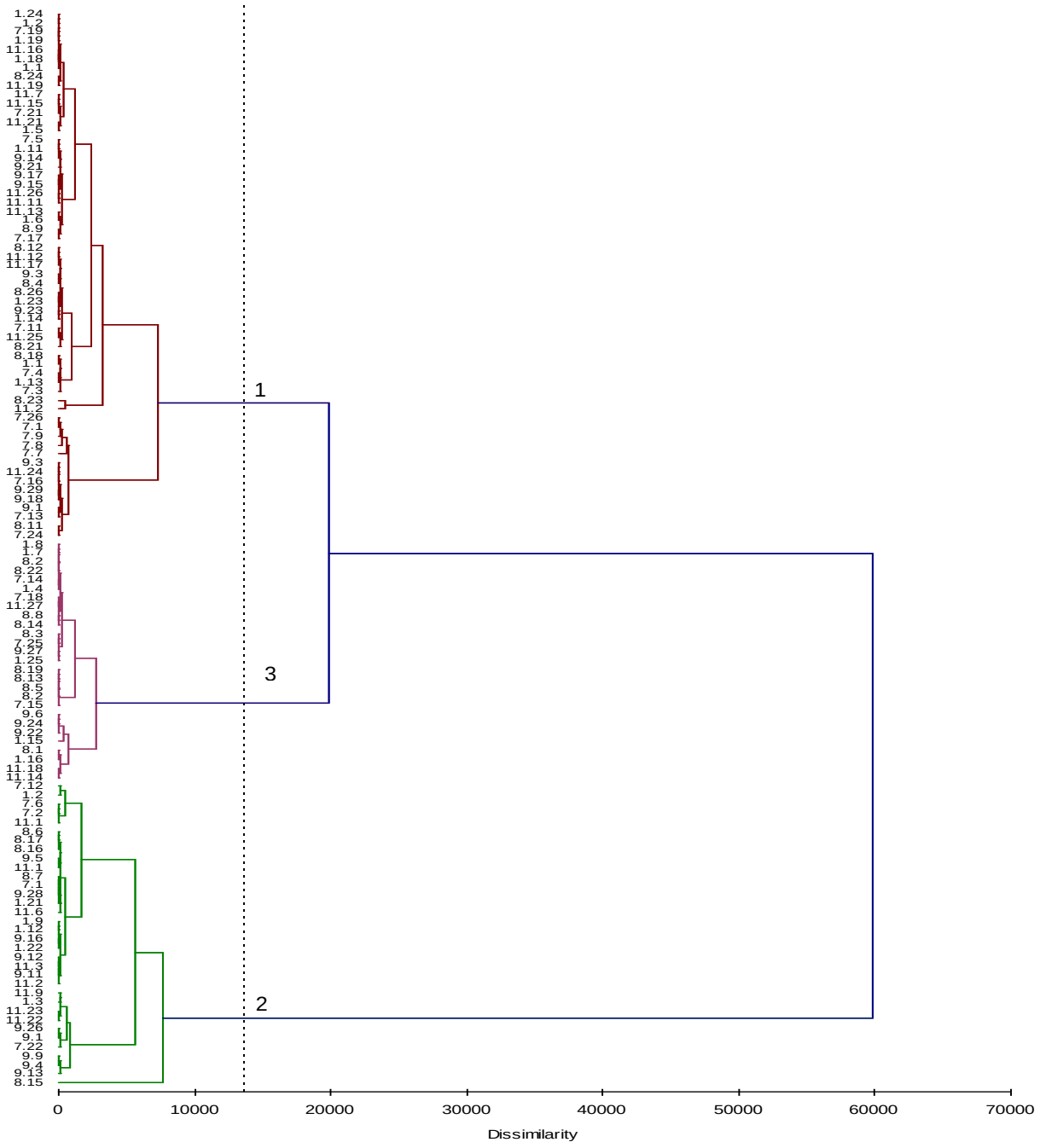

Figure 2. Dendrogram of the lines of *Medicago polymorpha* clustered based on Euclidean distances of dissimilarity matrix with the Ward's method. Enfidha (1), El Kef (7), Soliman (8), Bulla Regia (9), and Mateur (11).

lines including 12 lines from Enfidha, 13 lines from Mateur, 15 lines from El Kef, 9 from Soliman, and 10 lines from Bulla Regia. A second group is constituted by 34 lines with 6 lines from Enfidha, 8 from Mateur, 5 from El Kef, 5 from Soliman, 10 from Bulla Regia. A third group was composed by 6 lines from Enfidha, 3 from Mateur, 4 lines of El Kef, 10 of Soliman, and 4 lines of Bulla Regia. Lines of the first and second groups showed the highest values of LS while those of the third group had the lowest level (Table 5). On the other hand, the lines of the second group exhibited the highest NL while the lowest values were recorded for lines of the first and third groups. Studied populations were clustered into three groups (Figure 3). A first group was formed by Enfidha population, a second group included the population of El Kef, and a third group was constituted by

Table 5. Means of measured traits for classes of lines and populations of *Medicago polymorpha*.

Class\Variable	Lines						Populations					
	1	2	3	λ	F	p-value	1	2	3	λ	F	p-value
D1L	9.13	9.10	9.56	-	-	-	9.75[a]	8.96[b]	9.13[b]	0.941	3.678	0.028
FLOR	55.04	58.98	45.98	-	-	-	50.67	60.28	53.21	-	-	-
LS	36.47[a]	38.74[a]	26.29[b]	0.75	19.56	< 0.0001	-	-	-	-	-	-
NR	2.89	2.83	1.66	-	-	-	2.32	3.58	2.36	-	-	-
NL	65.53[b]	104.08[a]	35.88[c]	0.19	244.39	< 0.0001	64.53	70.99	71.12	0.991	0.547	0.580
AFW	-	-	-	-	-	-	3.11	4.15	3.09	-	-	-
ADW	0.73	1.01	0.33	-	-	-	0.59[b]	1.09[a]	0.64[b]	0.833	11.689	< 0.0001
RDW	-	-	-	-	-	-	0.04	0.15	0.33	-	-	-
ADWh2	1.15	3.96	0.79	-	-	-	0.95	1.68	2.23	-	-	-
NPOD	-	-	-	-	-	-	24.16[a]	15.23[b]	23.62[a]	0.855	9.895	0.000
WPOD	-	-	-	-	-	-	0.88	0.71	1.10	-	-	-
W100P	-	-	-	-	-	-	4.16	4.79	13.33	-	-	-

λ: Lambda of Wilks F: coefficient of Snedecor-Fisher. Days from emergence to first true leaf (D1L days); days from emergence to first flower (FLOR days); length of stems (LS cm); number of ramifications (NR); number of leaves (NL); aerial fresh weight (AFW g); aerial dry weight (ADW g); root dry weight (RDW g); aerial dry weight at second harvest (ADWh2 g); number of pods (NPOD); weight of pods (WPOD g); weight of 100 pods (W100P g). Means of each trait followed by the same letters are not significantly different between the three lines and populations' groups.

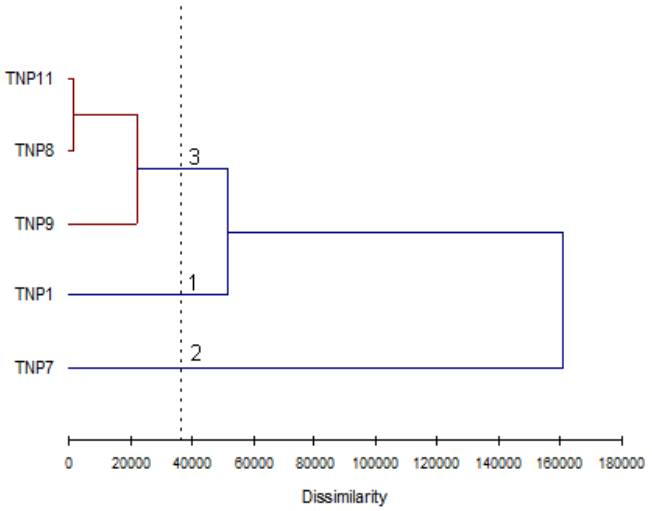

Figure 3. Dendrogram of populations of *Medicago polymorpha* clustered based on Euclidean distances of dissimilarity matrix with the Ward's method. Enfidha (TNP1), El Kef (TNP7), Soliman (TNP8), Bulla Regia (TNP9), and Mateur (TNP11).

Soliman, Bulla Regia and Mateur populations. The populations of first group showed the highest number of days from emergence to first true leaf (D1L) while the populations of second group showed the highest ADW (Table 5). Furthermore, the populations of first and third groups exhibited the highest NPOD.

Correlations between morphological traits and environmental parameters

Among the 55 correlations between the traits and environmental parameters, 9 correlations were significant and 6 of them were negative (Table 6). The altitude was positively correlated with FLOR, AFW and ADW while it was negatively correlated with NPOD. Furthermore, the temperature was negatively correlated with FLOR and ADW. The relative humidity was negatively associated with FLOR, AFW and RDW.

Table 6. Correlations between measured traits and environmental parameters for natural populations of *Medicago polymorpha*.

	Altitude	EC	Ann rain	T	RH
D1L	-0.14	-0.08	-0.10	0.17	0.16
FLOR	0.42*	-0.05	0.00	-0.36*	-0.40*
NR	0.26	-0.09	-0.06	-0.21	-0.16
NL	0.12	0.01	0.06	-0.08	-0.23
AFW	0.43*	-0.16	-0.10	-0.29	-0.39*
ADW	0.48*	-0.05	-0.10	-0.43*	-0.40*
RDW	-0.06	0.00	0.07	0.00	0.17
ADWh2	-0.04	0.01	0.07	-0.02	0.13
NPOD	-0.34*	0.14	0.13	0.32	0.07
WPOD	-0.09	0.00	0.06	0.04	0.18
W100P	-0.07	0.01	0.07	0.01	0.17

*Significant after using Bonferroni correction (α= 0.05/ 55 = 0.0009090909). Days from emergence to first true leaf (D1L days); days from emergence to first flower (FLOR days); number of ramifications (NR); number of leaves (NL); aerial fresh weight (AFW g); aerial dry weight (ADW g); root dry weight (RDW g); aerial dry weight at second harvest (ADWh2 g); number of pods (NPOD); weight of pods (WPOD g); weight of 100 pods (W100P g). Electro-conductivity (EC); mean annual rainfall (An rain); temperature (T); relative humidity (RH).

DISCUSSION

One hundred and twenty lines of *M. polymorpha*, sampled from different eco-geographical regions in Tunisia, were used to quantify phenotypic diversity. Sixteen vegetative and reproductive characters were measured to generate valuable information on genetic variation within and among populations of this species. Results from ANOVA support that the variation of the traits was explained by population and lines nested within population effects. The largest effect was recorded for population. Decomposition of variation within and among populations indicated that most differentiation was recorded within populations. Such a result is not expected with regard to the self-fertilizing mating system of *M. polymorpha*. Accordingly, highest phenotypic variance within populations were reported for natural Tunisian populations of *M. truncatula*, *M. laciniata* and *M. ciliaris* (Badri et al., 2007, 2008, 2010; Arraouadi et al., 2009) and for *Brachypodium distachyon* (Neji et al., 2014). Friesen et al. (2014) detected evidence of substantial migration between all pairs of Tunisian populations of *M. truncatula*. We expect that migration occurs largely by migration of seed pods in the wool or hair of animals.

In the current study, a substantial variation was detected among populations for 11 of the 16 investigated traits. These differences between populations were essentially related to flowering time, plant vigor, and biomass and pods production. This agrees with previous studies which revealed that *M. truncatula*, *M. laciniata* and *M. ciliaris* were highly variable for morpho-phenological characters (Badri et al., 2007, 2008, 2010, 2015; Arraouadi et al., 2009). The seed hardness and dormancy of seeds of *M. polymorpha* coupled with highly variable flowering time, allowed this species to survive unfavorable periods in a wide variety of bioclimatic zones. Among the 120 studied lines of *M. polymorpha*, there was no spineless line. Similarly, no spineless ecotype of this species has ever been found in Sardinia (Loi et al., 1995). Nevertheless, populations with non-spiny pods are very common in Chile (Del Pozo et al., 2002). This feature is of great interest to breeders and farmers, since the spiny pods of *M. polymorpha* are frequently caught in large numbers in sheep's hair, and drastically reduce the commercial value of the wool.

Our results showed that environmental variance was higher than genetic variance for most traits and consequently had a relatively low average of heritability. In addition, low levels of Q_{ST} were registered for most investigated characters. There was no significant association between population differentiation and geographical distances. These results are consistent with previous findings showing an absence of significant correlation between geographical distance and population differentiation in annual *Medicago* species (Badri et al., 2008, 2010) and *Brachypodium hybridum* (Neji et al., 2014). Nevertheless, Neji et al. (2013) observed that geographical distance partially explained the genetic distance among populations of *Sulla carnosa*.

The flowering time was positively correlated with NL, AFW, and ADW. Indeed, the latest flowering plants invest most of their effort in the aerial growth. These results are consistent with those found in natural populations of *M. truncatula*, *M. laciniata* and *M. ciliaris* (Badri et al., 2007, 2008, 2010). The induction of flowering is a central event in the life cycle of plants. Flowering is controlled by environmental conditions and developmental regulation (Mouradov et al., 2002). The harvest index (HI) was negatively correlated with LS, NL, AFW, ADW, and ADWh2, indicating that plants allocated higher effort to the vegetative growth produce lower number of pods. Clustering of studied lines into three groups differing in the LS and NL has implications with regard to choice of parents for creating segregating populations so as to maintain genetic diversity in a breeding program.

Most correlations were found between the altitude and measured traits followed by relative humidity and temperature, suggesting that the degree of trait variation differs according to the region where seeds were collected. Accordingly, Del Pozo et al. (2002) demonstrated that days to first flower were positively correlated with both latitude and longitude, as well as mean annual precipitation of collecting site of 69 Chilean accessions of *M. polymorpha*. Ecotypic differentiation related to environmental parameters along an aridity gradient in Syria or in a heterogeneous area of distribution such as Sardinia have been found in numerous annual legumes, for traits such as flowering time and degrees of hard seededness (Ehrman and Cocks, 1990; Piano et al., 1996) and shoot growth (Loi et al., 1993; Prosperi et al., 1991; Ovalle et al., 1993; Norman et al., 1998). Adaptive genetic variation is defined as the variation in genes that affects the fitness of an organism (Holderegger et al., 2006). Adaptation may be facilitated by co-adapted gene complexes, which are multi-locus genotypes favored by selection (Schemske, 2010).

In conclusion, our study demonstrates that morpho-phenological traits are useful tools for detecting variation within and among populations of *M. polymorpha*. Higher levels of variability for most traits were found to occur within populations. Our results suggest a prominent role for natural selection in accounting for patterns of genetic differentiation at quantitative traits among natural populations of *M. polymorpha.* Further study is needed to genotype lines of this species using simple sequence repeat (SSR) markers developed for the model legume *M. truncatula*. This will radically improve genetic characterizations and breeding programs of *M. polymorpha*.

Conflict of interests

The authors have not declared any conflict of interest.

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