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MORPHOLOGICAL AND PHENOLOGICAL VARIABILITY WITHIN SEVEN OASIS POPULATIONS OF PERENNIAL ALFALFA (*MEDICAGO SATIVA* L.) IN HYPER-ARID ZONE OF SOUTHERN ALGERIA

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Abstract

In Algerian Saharian oases, the surfaces reserved for local populations of perennial alfalfa (*Medicago sativa* L.) have declined significantly in favor of other introduced cultivars. Their preservation has become more than necessary. In this perspective, a study conducted over two successive campaigns, in an hyper-arid area of Algeria (Adrar region), focused on the expression of twenty seven (27) morphological and phenological characteristics related to plants, pods and seeds within seven (07) local populations of different provenances: El-Menea (Ghardaïa), Tamentit (Adrar), Timimoun (Timimoun), El Guerrara (Ghardaïa), Aoulef (Adrar), In Ghar (In Salah) and Temacine (Touggourt). The ecological factors (rainfall, temperature, altitude) of the alfalfa provenance environments were also considered. The trial was carried out in a randomized complete block design with four (04) replications. The results indicate the existence of a great morphological variability between the populations for traits related to flowering and pods. The study revealed very strong correlations, particularly between the number of pods per plant (NPPl), the number of seeds per plant (NSPl) and the number of flowers per plant (NFPl) during the two successive years. The rainfall and the temperature (provenance environmental factors) acted on almost the totality of the studied characteristics, whereas the effect of the altitude seems to be of less importance. The three factors (respectively, temperature, rainfall and altitude) would have an influence on the precocity of the populations Aoulef, Inghar, Tamentit and El menea which showed a better performance for seed number, in spite of the very difficult climatic conditions characterizing this region of the country.

Key words: Ecological factors; Fodder; Hyper-aridity; Legumes; Sahara; Variability.

Introduction

Perennial alfalfa (*Medicago sativa* L., *Fabaceae*) is one of the most widespread forage species in the world (Radovič *et al*., 2009; Li & Brummer, 2012; Hamd Alla *et al*., 2013; Ben Rhouma *et al*., 2017; Lorenzo *et al*., 2019). It is native to the central Middle East, which includes Iran, Transcaucasia, Asia Minor and the high regions of Turkmenistan (Boelt *et al*., 2015; Albayrak *et al*., 2018), and has become sub-cosmopolitan (Quezel & Santa, 1962; Albayrak *et al*., 2018). In Algeria, perennial alfalfa is a very common plant, except in the tell and the coast (Quezel & Santa, 1962).

Perennial alfafa is one of the most important forage species due to its high protein content, high digestibility and adaptation to different environmental conditions (Albayrak *et al*., 2018; Farshadfar, 2017; Mowaed, 2016; Radovič *et al*., 2009) with minimal inputs (Thiébau *et al*., 2003; Radovič *et al*., 2009). Thanks to its high protein content and digestibility (Radovič *et al*., 2009; Mielmann, 2013; Albayrak *et al*., 2018), alfalfa is a valuable feed for all categories of animals (especially ruminants) (Suttie, 2004); it participates indirectly in human nutrition through meat and milk consumption (Riasat *et al*., 2020).

More than that, thanks to its deep-rooting system and its perenniality, Alfalfa (*Medicago sativa* L) ensures the economic sustainability of cropping systems (Tlahig *et al*., 2017), improving the soil and limiting its erosion (Li & Brummer, 2012).

It is also a perennial legume that can fix its own nitrogen in association with rhizobia bacteria (Butler *et al*., 2018), provides nitrogen to the plant and increases soil N fertility for subsequent crop rotations (Li & Brummer, 2012; Kumar *et al*., 2017).

Furthermore, a large genetic variability exists within the *Medicago sativa* complex (Julier *et al*., 1995; 2000; Mauriès, 2003) due mainly to natural and anthropogenic selection in different climates and localities (Touil *et al*., 2008). This genetic variability gives it a high adaptability to different environmental conditions (Bolaños *et al*., 2000; Ahsyee *et al*., 2013; Kumar *et al*., 2017; Costea *et al*., 2019). To study this genetic diversity, several authors have used different agronomic and morphological criteria (Dehghan-Shoar *et al*., 2005; Loumerem *et al*., 2007; Basafa & Taherian, 2009; Al-Faifi *et al*., 2013; Arab *et al*., 2015; Monirifar & Abdollahi, 2014; Tlahig *et al*., 2017; Chabouni *et al*., 2019). These criteria are often used in breeding programmes to develop high-yielding cultivars (quantitative and qualitative) (Bolaños *et al*., 2000; Julier *et al*., 2000, 2014; Badr *et al*., 2020).

In the Saharian regions, perennial alfalfa was once the most widely grown fodder legume (Chaabena & Abdelguerfi, 2001). Over time, farmers have replaced these local populations of perennial alfalfa with introduced commercial varieties, assumed to be high-yielding (Benrouhma *et al*., 2017), which ultimately have little or no adaptation to local conditions (Tirichine & Allam, 2016). This has led to an erosion of genetic diversity and sometimes even extinction of local populations (Benabderrahim *et al*., 2015).

The present contribution is made within the framework of the evaluation and preservation of plant genetic resources of fodder and pastoral interest in Algeria, particularly in the Saharian zone. The aim of this study is to evaluate seven local populations of perennial alfalfa in the hyper-arid conditions of the Adrar region, considered as one of the hottest regions in the world, through the expression of certain morphological and phenological characteristics (vegetative development, flowering, pods and seeds).

This work follows the numerous studies carried out on forage legumes in northern and southern Algeria (Alane *et al*., 2017; Bouaboub, 2001; Bouziane *et al*., 2019, 2023; Chaabena *et al*., 2004, 2011; Chabouni *et al*., 2019; Issolah *et al*., 2006, 2007, 2011, 2012, 2014, 2015, 2016, 2022).

Material and Methods

Plant material and experimental setup: Following the survey mission conducted in various regions of southern Algeria, seven local Saharan populations of perennial alfalfa were collected from seed farmers at different localities, during the year 2016: El-Menea (Ghardaïa), Tamentit (Adrar), Timimoun (Timimoun), El Guerrara (Ghardaïa), Aoulef (Adrar), In Ghar (In Salah) and Temacine (Touggourt) (Fig. 1 & Table 1).The populations of El-Menea (introduced in Adrar region from the zone of Ghardaïa) and Tamentit (cultivated for a long time in the palm groves of "Touat", central Adrar) are the only ones still cultivated in Adrar region (south-west Algeria). The Temacine population is the only one that comes from the south-east of Algeria (Touggourt, zone of Ouargla). All seven populations come from localities characterized by a desert climate: there is almost no rainfall throughout the year (Table 1).

The trial was conducted for two successive years (2016/2017 and 2017/2018) on a plot located at **the INRAA experimental station in Adrar (**Fig. 1), at an altitude of 253 m. The climate of the Adrar region obeys the general characteristics of the Saharian climate with a cold winter sometimes reaching 0°C (December-January), while in summer the temperature can exceed 49°C (July-August); frosts are rare but can be recorded, even causing catastrophic damage (Alane *et al*., 2017; Boulgheb, 2017; Tayebi & Khelafi, 2021). The soil of the experimental plot has a coarse sandy-silty texture; the pH is slightly alcaline (7.73); the electrical conductivity is quite high (Boulgheb, 2017). The seven populations, each represented by 30 individuals, were shown on November 6th, 2016. The trial was carried out in a randomized complete block design with 4 replications under localised irrigation (drip), uniformly for the entire plot. The irrigation conditions are identical for the 4 blocks.

Morphological characteristics

Globally, twenty-seven (27) variables were considered.

Twelve (12) characteristics related to the vegetative development of the plant: Seed germination capacity **(GCS)**; emergence date **(ED)**; number of branches per plant **(NBP)**; stem diameter **(SD)**; number of leaves per plant **(NLPl)**; number of leaves per stem **(NLS)**; winter stem height **(WSH)**; spring stem height **(SSH)**; fifth leaf length **($L5^{th}l$)**; fifth leaf width **($W5^{th}l$)**; sixth leaf length **($L6^{th}l$)** and sixth leaf width **($W6^{th}l$)**.

Eleven (11) characteristics related to flowering and production: Appearance date of first flower **(D1F)**; number of flowers per inflorescence **(NFI)**; number of flowers per plant **(NFP)**; end date of flowering **(EFD)**; appearance date of first green pod **(GPD)**; appearance date of first ripe pod **(MPD)**; number of pods per plant **(NPPl)**; number of seeds per pod **(NSP)**; number of seeds per plant **(NSPl)**; seed diameter **(ØS)** and 1000-seed weight **(WTS)**.

Four (04) characteristics were deduced: Winter growth rate **(WGR)**; spring growth rate **(SGR)**; flowering duration **(FD)** and plant cycle length **(CL)**.

Statistical analysis

The data obtained during the two successive campaigns were subjected to a variance analysis supported by a Tukey HSD post-hoc analysis when the ANOVA was significant, correlation matrix and a principal component analysis of the variables. Data relating to the ecological factors (altitude, temperature, rainfall) of the provenance areas, of the different populations, were also considered. Statistical processing was carried out using Minitab (2003) and Xlstat (2013) softwares.

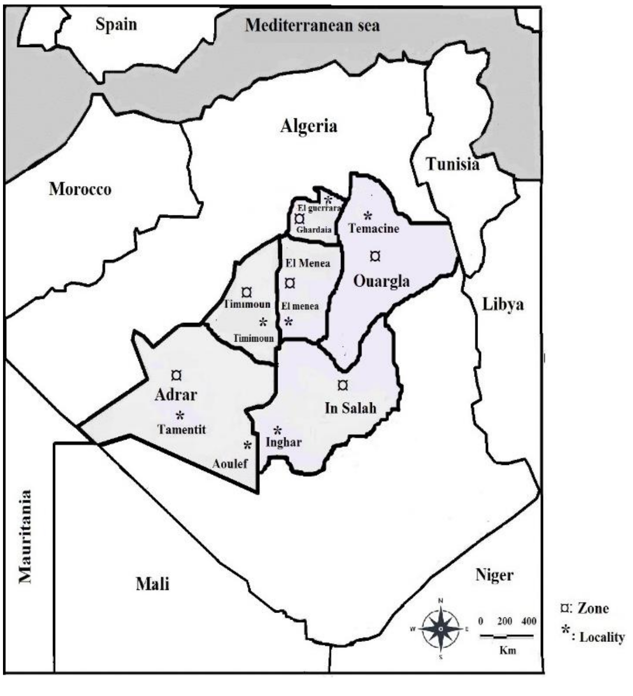

Fig. 1. Provenance areas of seven local populations of perennial alfalfa in Algeria.

Table 1. Ecological characteristics of the provenance areas of seven local populations within perennial alfalfa in Algeria.

Population name	provenance area	Altitude (m)	Climate	T (°C)	Tmax (°C)	Tmin (°C)	Rainfall (mm/an)
El-menea	South-western Algerian Sahara (Ghardaïa)	397	Arid	23,10	30,25	15,00	37,62
Tamentit	South-western Algerian Sahara (Touat, Adrar)	240	Hyper-arid	26	34	18	13,947
Temacine	South-eastern Algerian Sahara (Touggourt, Ouargla)	84	Arid	22,74	29,61	15,73	50,11
Timimoun	South-western Algerian Sahara (Gourara, Timimoun)	300	Hyper-arid	25,12	32,38	17,35	16,42
El-Guerrara	South-West Algerian Sahara (Ghardaïa)	350	Arid	22,75	28,66	16,70	65,07
Aoulef	South-western Algerian Sahara (Tidikelt, Adrar)	290	Hyper-arid	27	34	19	10
In Ghar	South-Western Algerian Sahara (In Salah)	260	Hyper-arid	27	34	19,42	6,35

Sources**:** (Chabouni, 2019; DGF, 1998; https://fr.tutiempo.net/climat/algerie.html.)

T: Mean monthly temperature; **Tmax**: Mean maximum temperature**, Tmin**: Mean minimum temperature; rainfall: Mean annual precipitation (mm). Data determined over a period of 10 years (2010-2019)

Table 2. Results of the variance analysis within seven oasis populations of perennial alfalfa in Algeria during the two years of study.

Variables*	Min*		Max*		Mean*		Standard deviation		Probability	
	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
GCS	99	98	100	100	91	90	28	28	-	-
ED (days)	4	5	14	19	6	11	1.60	1.60	0.000***	0.000***
NBP	2	3	18	18	13	13	3	3	0.000***	0.000***
SD (cm)	0.24	0.24	3.00	0.59	0.37	0.37	0.11	0.05	0.023*	0.000***
NLPl	10	8	1020	846	326	305	155	140	0.000***	0.000***
NLS	3	2	63	60	24	23	9	9	0.000***	0.000***
WSH (cm)	6	10	110	120	50	61.38	19.53	19.19	0.000***	0.000**
SSH (cm)	20	12	155	189	90	84.15	21	21.40	0.000***	0.000***
L5thl (cm)	1.6	1.5	4.6	4.6	2.8	2.80	0.51	0.51	0.000***	0.000***
W5thl (cm)	1.6	1.0	4.5	4.5	2.9	2.90	0.52	0.51	0.000***	0.000***
L6thl (cm)	1.6	1.4	4.3	4.3	2.67	2.72	0.45	0.48	0.000***	0.000***
W6thl (cm)	1.7	0.9	4.3	4.3	2.8	2.72	0.48	0.48	0.000***	0.000***
D1F (days)	72	48	206	147	133	72	26.22	19	0.000***	0.000***
NFI	2	1	20	25	10	8	4	4	0.000***	0.000***
NIPl	1	1	80	77	17	15	9	10	0.000***	0.000***
NFPl	2	3	1120	964	188	132	147	123	0.000***	0.000***
EFD (days)	164	167	273	226	208	177	29	70.94	0.000***	0.000***
GPD (days)	120	59	226	157	157	93	26	24	0.000***	0.000***
MPD (days)	139	98	248	183	184	138	29	14	0.000***	0.000***
NPPl	2	2	1120	918	184	125	151	117	0.000***	0.000***
NSP	1	1	9	8	5	4	2	1.37	0.000***	0.02*
NSPl	8	8	5760	3948	853	442	706	422	0.000***	0.000***
ØS (mm)	1.00	1	2.2	2	1.38	1.37	0.22	0.22	0.000***	0.000***
WTS (g)	2.5	2.4	3.6	3.4	2.65	2.78	0.37	0.32	0.000***	0.000***
WGR(cm/days)	0.06	0.17	1.16	1.94	0.53	1.01	0.21	0.31	0.000***	0.000***
SGR (cm/days)	0.12	0.09	1.19	1.47	0.57	0.65	0.13	0.17	0.000***	0.000***
FD (days)	28	61	251	126	76	101	21	12	0.000***	0.000***
CL (days)	185	158	294	251	229	198	29	14	0.000***	0.000***

Min: Mean of a population with the lowest value, Max: Mean of a population with the highest value, Mean: Mean of the species. Probability: : *p≤0, 05 ; **p≤0, 01 and *** p≤0, 001.

(ED): emergence date; **(NBP):** number of branches per plant; **(SD):** stem diameter; **(NLPl):** number of leaves per plant; **(NLS):** number of leaves per stem; **(WSH):** winter stem height; **(SSH):** spring stem height; **(L5thl):** length of fifth leaf; **(W5thl):** width of fifth leaf; **(L6thl)** and **(W6thl):** length of sixth leaf and width of sixth leaf; **(D1F):** appearance date of first flower; **(NFI):** number of flowers per inflorescence; **(NIPl**): number of inflorescence per plant; **(NFPl):** number of flowers per plant; **(EFD):** end of flowering date; **(GPD):** 1st green pod date; **(MPD):** 1st mature pod date; **(NPPl):** number of pods per plant; **(NSP):** number of seeds per pod; **(NSPl):** number of seeds per plant; **(ØS):** seed diameter; **(WTS):** 1000 seed weight; **(WGR):** winter growth rate; **(SGR):** spring growth rate; **(FD):** flowering duration; **(CL):** cycle length; **(GCS)**: germination capacity of seeds

Results and Discussion

Variance analysis (ANOVA): The variance analysis indicates that the variability between the oasis populations is quite marked since the differences are highly to very highly significant for most of the studied traits for the two successive seasons (Table 2).

Highly to very highly significant differences were noted for pod and seed traits. The Aoulef and In Ghar populations were distinguished from the other populations by their higher pod and seed numbers. The Tukey test shows the clear distinction of these two populations (Aoulef and In Ghar) with regard to the number of pods per plant (NPPl), the number of seeds per pod (NSP) and the number of seeds per plant (NSPl) (Table 3). The Timimoun population showed the highest 1000-seed weight (1000-SW) in both years of the trial.

Prosperi *et al.*, (1993)[a] note, however, that the late ecotypes in perennial alfalfa often result in a longer cycle and a decrease in pod production. This was observed in our trial (both seasons) for the Timimoun, Guerrara and Temacine populations, which are the latest compared to the other studied populations. The same behaviour was observed by Chabouni *et al.*, (2019) for the Temacine population compared to El-Menea and Tamentit populations during a behavioural study conducted under the same edapho-climatic conditions. Furthermore, Prosperi *et al.*, (1993)[b] noted that in the genus *Medicago*, early ecotypes have a shorter cycle.

Our results also indicate clear differences for flowering traits in the two years of the study. The Tukey test shows this distinction (Table 3).

The obtained results show, respectively, a clear precociousness of Aoulef, Inghar, Tamentit and El menea populations, which occurred in both years. These populations also recorded a greater number of flowers per inflorescence (NFI) and per plant (NFPl) than the other later populations: El Guerrara, Timimoun and Temacine (Table 3).

The maximum flowering duration (FD) is observed on In Ghar during the second year, while the minimum is observed in El Guerrara, Timimoun and Temacine (Table 3). In the *Medicago* genus, Yahiaoui & Abdelguerfi (1995, 1999) indicate that annual populations (*Medicago orbicularis* (L.) Bart and *medicagociliaris*) with good vegetative development seem to be the latest to flower and, at the same time, have a shorter flowering period.

On the other hand, the study conducted by Chabouni *et al*., (2019), on perennial alfalfa populations in the hyper-arid climatic conditions of southern Algeria (Adrar region), showed that the earliest population (El Menea), had a higher number of flowers per inflorescence (NFI) and per plant (NFPl), in contrast to Temacine which is the latest. Nevertheless, the latter showed a good vegetative development (Chabouni *et al*., 2019).

Overall, in the light of this study, we can establish that the local alfalfa populations seem to have a similar and stable behaviour, from one year to another, despite the existence of an important morphological diversity (very significant variation) within and between the populations during the same year.

Synthesis of correlations: The analysis of the correlation matrix results reveals many significant relationships between the different traits for the two successive years (Table 4).

For the traits related to vegetative development, the number of stem branches (NBPl) has a very significant influence on the number of leaves per stem (NLS) and per plant (NLPl) for the first and second year of the study. In addition, the number of leaves per stem (NLS) has a very significant influence on the number of leaves per plant (NLPl). According to Chabouni *et al*., (2019), as the plant branches, there is an increase in the number of leaves on the stems and plant; the In Ghar and Tamentit populations, which branched the most, showed a large number of leaves per stem (NLS) and per plant (NLPl) compared to the other populations.

The same results were obtained by Chaabena *et al*., (2004) and Chabouni *et al*., (2019) on the behaviour of Saharan populations of *Medicago sativa* in southern Algeria.

Concerning the flowering and reproductive traits: the appearance date of the first flower (D1F) during the two years (Table 4) is very strongly correlated with the number of flowers per plant (NFPl), the number of pods per plant (NPPl) and the number of seeds per plant (NSPl).

We noted that the precocious populations (Aoulef, In Ghar, Tamentit and El-menea) have high flower and seed numbers. Chaabena *et al*., (2004) and chabouni *et al*., (2019) obtained the same results on saharian populations of perennial alfalfa. According to Boelt *et al*., (2015), there is a strong genetic correlation in perennial alfalfa between seed yield per plant and some components: number of inflorescences and flowers per plant, number of seeds per plant and seed weight per inflorescence. According to Bolaños-Aguilar *et al*., (2000) and Bodzon (2004), the inflorescence components are an important criterion in the selection of plants with high seed production. Furthermore, there is a strong correlation between the number of stem branches (NBPl) (traits related to vegetative development), flower and seed yield (NFI; NFPl; NPPl and NSPl) in both years. The same observation was made by Abadouz *et al*., (2010). This correlation of stems number with the seeds number per plant could represent a very good criterion for selecting plants with a high seed yield potential (Bodzon, 2004). On the other hand, the study of the correlations between the different morphological characteristics and the altitude, rainfall and temperature corresponding to the provenance sites of *M. sativa* populations, showed the relationship existing between the morphology of *M. sativa* and the environmental conditions (Table 5).

Table 3. Tukey HSD post-hoc analysis (at 95% confidence level) of flowering, pod and seed traits within seven oasis populations of perennial alfalfa in Algeria during the two years of study.

Variables populations	D1F (days)		NFI		NFPl		FD (days)		NPPl		NSP		NSPl	
	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
El-menea	123^B	67^B	11^B	8^B	178^B	123^B	84^A	104^B	171^B	123^B	5^A,B	4^A,B	828^B	456^B
Tamentit	112^C	63^B	13^A	10^A	255^A	188^A	72^B	102^B	249^A	188^A	5^A,B	4^A,B	1133^A	604^A,B
Temacine	157^A	84^A	8^C	6^C	115^C	73^C	78^A,B	95^C	110^C	73^C	4^B	3^B	452^C	211^C
Timimoun	152^A	88^A	8^C	6^C	105^C	71^C	76^A,B	95^C	101^C	71^C	4^B	4^A,B	456^C	240^C
El-Guerrara	158^A	87^A	8^C	6^C	106^C	70^C	76^A,B	95^C	104^C	70^C	4^B	4^A,B	448^C	235^C
Aoulef	111^C	62^B	13^A	10^A	296^A	215^A	73^B	103^B	288^A	215^A	5^A,B	4^A,B	1320^A	677^A
In Ghar	116^C	55^C	12^A,B	8^B	256^A	173^A	73^B	111^A	259^A	73^A	5^A	4^A	1305^A	644^A

(D1F): appearance date of the first flower; **(NFI)**: number of flowers per inflorescence; **(NFPl)**: number of flowers per plant; **(FD)**: flowering duration; **(NPPl)**: number of pods per plant; **(NSP)**: number of seeds per pod; **(NSPl)**: number of seeds per plant;
The letters A, B, C correspond to the homogeneous groups.

Table 4. Correlation of traits within seven oasis populations of perennial alfalfa in Algeria during two years.

	ED (days)		NBPl		SD (cm)		NLS		NLPl		D1F (days)		NFI		NFPl	
	1st year	2nd year	1st year	2nd yeare	1st year	2nd year	1st year	2nd year	1st year	2nd yeare	1st year	2nd year	1st year	2nd yeare	1st year	2nd year
NBPl	-0.001	0.017														
	0.972	0.670														
SD (cm)	-0.012	0.023	0.025	-0.017												
	0.739	0.555	0.498	0.670												
NLS	0.033	0.049	0.330	0.266	0.004	0.085	-									
	0.364	0.211	0.000***	0.000***	0.902	0.029*										
NLPl	0.028	0.047	0.863	0.833	0.005	0.040	0.655	0.684	-							
	0.434	0.226	0.000***	0.000***	0.894	0.307	0.000***	0.000***								
D1F (days)	0.132	0.122	0.589	-0.300	0.113	-0.044	0.312	-0.206	0.502	-0.327	-					
	0.000***	0.002***	0.000***	0.000***	0.002***	0.259	0.000***	0.000***	0.000***	0.000***						
NFI	-0.134	-0.153	0.306	0.326	0.059	0.095	0.134	0.109	0.233	0.271	0.500	-0.215				
	0.000***	0.000****	0.000***	0.000***	0.101	0.014*	0.000***	0.005**	0.000***	0.000***	0.000***	0.000***				
NFPl	0.125	-0.145	0.487	0.459	0.077	0.164	0.218	0.213	0.394	0.424	0.527	0.258	0.682	0.687		
	0.001***	0.000***	0.000***	0.000***	0.034*	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***		
GPD (days)	0.142	0.043	0.614	-0.201	-0.084	-0.069	-0.290	-0.196	-0.521	-0.257	0.884	0.869	-0.517	-0.160	-0.549	-0.193
	0.000***	0.287	0.000***	0.000***	0.020*	0.075	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
NPPl	-0.125	-0.145	0.487	0.459	0.077	0.164	0.217	0.213	0.394	0.424	-0.527	-0.258	0.682	0.687	1.000	1.000
	0.001***	0.000***	0.000***	0.000***	0.034*	0.000***	0000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
NSP	-0.026	-0.052	0.025	-0.037	-0.051	-0.067	0.025	-0.016	0.034	-0.040	-0.062	-0.076	-0.080	-0.176	-0.130	-0.208
	0.473	0.195	0.487	0.345	0.155	0.087	0.488	0.684	0.354	0.355	0.086	0.045*	0.026*	0.000***	0.000***	0.094
NSPl	-0.156	-0.183	0.449	0.393	0.063	0.148	0.206	0.197	0.363	0.365	-0.524	-0.277	0.606	0.575	0.893	0.840
	0.000***	0.000***	0.000***	0.000***	0.081	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***

Content of the cell : Pearson correlation (r); p-value

Probability: *$p \leq 0{,}05$; **$p \leq 0{,}01$ and *** $p \leq 0{,}001$.

(ED): emergence date; **(NBPl)**: number of branches per plant; **(SD)**: stem diameter; **(NLS)**: number of leaves per stem; **(NLPl)**: number of leaves per plant; **(D1F)**: date of first flower appearance; **(NFI)**: number of flowers per inflorescence; **(NFPl)**: number of flowers per plant; **(GPD)**: 1st green pod date ; **(NPPl)**: number of pods per plant; **(NSP)**: number of seeds per pod; **(NSPl)**: number of seeds per plant

Table 5. Relationship of biometric traits and ecological factors of the provenance areas within seven oasis populations of perennial alfalfa in Algeria for two years.

Factors / Traits	Altitude		Temperature		Rainfall	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
ED (days)	-0.103 **0.004****	-0.058 0.133	-0.161 **0.000*****	-0.175 **0.000*****	0.155 **0.000*****	0.165 **0.000*****
NBP	0.016 0.662	0.023 0.554	0.445 **0.000*****	0.440 **0.000*****	-0.420 **0.000*****	-0.421 **0.000*****
SD (cm)	-0.074 **0.040***	-0.177 **0.000*****	0.065 0.070	0.098 **0.011***	-0.041 0.255	-0.080 **0.038***
NLPl	0.014 0.696	0.006 0.885	0.404 **0.000*****	0.446 **0.000*****	-0.370 **0.000*****	-0.410 **0.000*****
NLS	0.026 0.477	0.033 0.399	0.216 **0.000*****	0.212 **0.000*****	-0.171 **0.000*****	-0.160 **0.000*****
WSH (cm)	0.062 0.088	0.086 **0.027***	0.525 **0.000*****	0.528 **0.000*****	-0.464 **0.000*****	-0.471 **0.000*****
SSH (cm)	0.004 0.918	0.048 0.216	0.259 **0.000*****	0.245 **0.000*****	-0.251 **0.000*****	-0.238 **0.000*****
L5thl (cm)	-0.029 0.427	-0.058 0.134	-0.302 **0.000*****	-0.341 **0.000*****	0.259 **0.000*****	0.310 **0.000*****
W5thl (cm)	-0.013 0.726	-0.017 0.659	-0.147 **0.000*****	-0.140 **0.000*****	0.111 **0.000*****	0.103 **0.008****
L6thl (cm)	0.022 0.541	0.005 0.902	-0.250 **0.000*****	-0.289 **0.000*****	0.215 **0.000*****	0.270 **0.000*****
W6thl (cm)	-0.011 0.751	-0.028 0.477	-0.180 **0.000*****	-0.230 **0.000*****	0.137 **0.000*****	0.194 **0.000*****
D1F(days)	-0.136 **0.000*****	-0.076 **0.050***	-0.606 **0.000*****	-0.472 **0.000*****	0.570 **0.000*****	0.448 **0.000*****
NFI	0.072 **0.048***	0.096 **0.013***	0.372 **0.000*****	0.363 **0.000*****	-0.331 **0.000*****	-0.322 **0.000*****
NIPl	-0.019 0.609	-0.012 0.762	0.355 **0.000*****	0.349 **0.000*****	-0.327 **0.000*****	-0.326 **0.000*****
NFPl	0.020 0.579	0.036 0.358	0.412 **0.000*****	0.380 **0.000*****	-0.370 **0.000*****	-0.342 **0.000*****
EFD (days)	-0.086 **0.017***	-0.013 0.730	-0.645 **0.000*****	-0.388 **0.000*****	0.596 **0.000*****	0.354 **0.000*****
GPD (days)	-0.095 **0.009****	-0.062 0.107	-0.663 **0.000*****	-0.395 **0.000*****	0.626 **0.000*****	0.384 **0.000*****
MPD (days)	-0.084 **0.019***	-0.013 0.733	-0.648 **0.000*****	-0.388 **0.000*****	0.599 **0.000*****	0.354 **0.000*****
NPPl	0.020 0.582	0.036 0.360	0.412 **0.000*****	0.380 **0.000*****	-0.370 **0.000*****	-0.342 **0.000*****
NSP	0.050 0.169	0.056 0.147	0.120 **0,001****	0.055 0.159	-0.122 **0.001****	-0.067 0.083
NSPl	0.044 0.223	0.074 0.056	0.438 **0,000*****	0.374 **0.000*****	-0.402 **0.000*****	-0.346 **0.000*****
ØS (mm)	0.083 **0.022***	0.098 0.011	0.011 0.755	0.031 0.418	-0.031 0.396	-0.054 0.162
WTS (g)	0.019 0.605	0.027 0.483	0.432 **0.000*****	0.439 **0.000*****	-0.495 **0.000*****	-0.500 **0.000*****
WGR (cm/days)	0.063 0.081	0.085 **0.029***	0.516 **0.000*****	0.514 **0.000*****	-0.456 **0.000*****	-0.458 **0.000*****
SGR (cm/days)	-0.001 0.983	0.045 0.241	0.252 **0.000*****	0.237 **0.000*****	-0.245 **0.000*****	-0.230 **0.000*****
FD (days)	0.052 0.147	0.106 **0.006****	-0.131 **0.000*****	0.316 **0.000*****	0.104 **0.004****	-0.316 **0.000*****
CL (days)	-0.086 **0.017***	-0.013 0.730	-0.645 **0.000*****	-0.388 **0.000*****	0.596 0.000***	0.354 **0.000*****

Content of the cell:
Pearson correlation (r)
P-value
Probability: *$p \leq 0,05$; **$p \leq 0, 01$ et *** $p \leq 0, 001$; Factors: Ecological factors of provenance areas.
(ED): emergence date; **(NBP)**: number of branches per plant; **(SD)**: stem diameter; **(NLPl)**: number of leaves per plant; **(NLS)**: number of leaves per stem; **(WSH)**: winter stem height; **(SSH)**: spring stem height; **(L5thl)**: length of fifth leaf; **(W5thl)**: width of fifth leaf; **(L6thl)** and **(W6thl)**: length of sixth leaf and width of sixth leaf; **(D1F)**: appearance date of first flower; **(NFI)**: number of flowers per inflorescence; **(NIPl)**: number of inflorescence per plant; **(NFPl)**: number of flowers per plant; **(EFD)**: end of flowering date; **(GPD)**: 1st green pod date; **(MPD)**: 1st mature pod date; **(NPPl)**: number of pods per plant; **(NSP)**: number of seeds per pod; **(NSPl)**: number of seeds per plant; **(ØS)** : seed diameter; **(WTS)**: 1000 seed weight; **(WGR)**: winter growth rate; **(SGR)**: spring growth rate; **(FD)**: flowering duration; **(CL)**: cycle length; **(GCS)**: germination capacity of seeds.

A very strong correlation with temperature and rainfall data was observed for most of the traits, particularly those related to flowering and reproduction. Populations that presented an early inflorescence appearance come from areas characterized by a high temperature and altitude with a weak rainfall. The obtained results lead us to conclude that the traits related to flowering and seeds are subject to the effect of temperature, rainfall and altitude, respectively. The precocity of the populations would be a form of escape from the unfavourable environmental conditions in the south of Algeria. Previous studies carried out on forage legumes (*Fabaceae*) in the north of Algeria, have shown that rainfall has a highly significant effect on flowering, particularly the beginning of flowering. Indeed, in the *Medicago* and *Trifolium genera*, results indicated that the precocious populations came from the least watered regions (Si Ziani & Abdelguerfi, 1995; Issolah & Abdelguerfi, 1998; Chebouti & Abdelguerfi, 1999; Issolah & Abdelguerfi, 1999).

Principal component analysis: Given the high number of variables and in order to obtain a clear illustration of the results corresponding to the two campaigns, only the most significant variables were taken into account. For the characteristics **related to vegetative development**, the number of stem branches (NBPl), the number of leaves per stem (NLS) and the number of leaves per plant (NLPl) were considered; for characteristics **related to flowering and reproduction**, the first flower appearance date (D1F), the number of inflorescences (NIPl) and flowers per plant (NFPl), the first green pod appearance date (GPD), the number of pods (NPPl) and seeds per plant (NSPl) and the number of seeds per pod (NSP) were considered.

The results revealed the existence of two goups of variables for which the seven populations differ the most during the two years (figures 2 and 3). The first one, includes most of the characteristics that are very positively correlated (NBP, NLS, NLPl, NFI, NIPl, NFPl, GPD, NPPl and NSPl); the second one, includes the characteristics D1stF and GDP; these two groups of variables are negatively correlated. Thus, the more the climate conditions are arid, the more the phenomenon of precocity is observed, as it is the case of the populations (Aoulef, In Ghar, Tamentit and El- menea) which seem to perform better on the vegetative level as well as on the level of flowers and seeds production compared to the populations (El Guerrara, Timimoun and Temacine), later and less performing, during the two years of the experiment. (Figs. 2 and 3).

In Algeria, a previous study carried out on three oasis populations of perennial alfalfa (El-menea, Tamentit and Temacine) showed the existence of a significant morphological variability for the most traits; El-menea population (the earliest), showed a better performance especially in terms of seed yield (Chabouni *et al*., 2019).

Studies conducted by Benabderrahim *et al*., (2009) and Tlahig *et al*., (2017) on Tunisian oasis populations of perennial alfalfa have demonstrated the existence of a very remarkable intra-population genetic diversity for most of the assessed morphological traits. Very interesting correlations were identified between some traits related to vegetative development (green biomass yield) and seed yield traits (flower and seed development) (Tlahig *et al*., 2017). These preliminary studies could be used to obtain high-yielding hybrid cultivars of perennial alfalfa under the arid conditions of Tunisian oases (salt stress, drought) (Benabderrahim *et al*., 2009; 2015 and Tlahig *et al*., 2017).

Other studies were based on the selection of perennial alfalfa (*Medicago sativa* L.) cultivars with high seed yield potential, focusing on its components (Askarian *et al*., 1995; Abadouz *et al*., 2010; Iannucci *et al*., 2002; Liatukiene *et al*., 2009; Sengul & Sengul, 2006; Bakheit *et al*., 2017). This criterion (seed yield) has little agronomic importance but is used to develop alfalfa cultivars with very good market value (Bolaños-Aguilar *et al*., 2000).

The present study highlighted even earlier local populations of perennial alfalfa (*Medicago sativa*), namely Aoulef, Tamentit, In Ghar and El-menea.

Other comparative studies have shown an earliness of local populations of perennial alfalfa compared to cultivars introduced in arid environments. Earliness seems to play a determining role in yield performance (high number of cuts) in addition to other important characteristics such as grain yield, forage quality, etc. (Chaabena *et al*., 2004; Bouaboub *et al*., 2008).

Paradoxically, in sub-humid environments, introduced populations of *Medicago sativa* are the most productive (Omari *et al*., 2017; Hadj Omar *et al*., 2018; Achir *et al*., 2020).

Based on the present results (hyper-arid region) obtained on seven local populations of *Medicago sativa* and those established in previous studies, conducted in Algeria on local oasis populations of perennial alfalfa, in relatively more favourable regions (arid and sub-humid regions) (Bouaboub, 2001; Chaabena & Abdelguerfi, 2001; Chaabena *et al*., 2004; Rahal- Bouziane, 2005; Bouaboub *et al*., 2008; Alane *et al*., 2017; Chabouni *et al*., 2019), we could thus reinforce the previously stated hypothesis (Chabouni *et al*., 2019) based on only three local populations, according to which the more pronounced the aridity conditions are, the more important the phenomenon of earliness and yield is in *Medicago sativa* L.

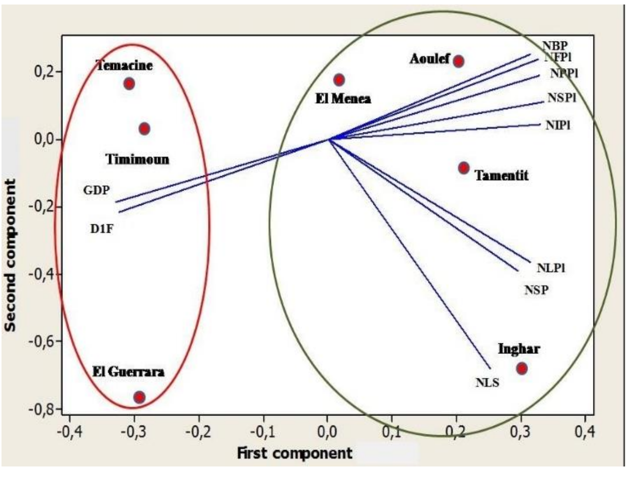

Fig. 2. Principal component analysis of variables related to vegetative development, flower and seed yield within seven local populations of perennial alfalfa in Algeria (1st year).

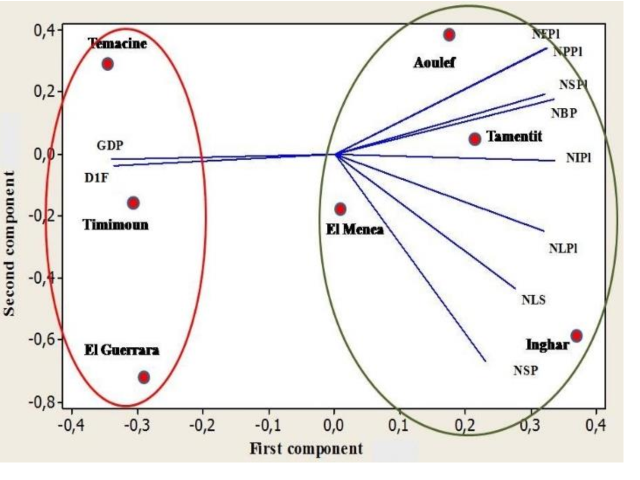

Fig. 3. Principal component analysis of variables related to vegetative development, flower and seed yield within seven local populations of perennial alfalfa in Algeria (2nd year).

Conclusion

This study carried out in a hyper-arid zone of Algeria (Adrar region), revealed the existence of a great morphological variability within the seven local populations of perennial alfalfa, expressed through highly to very highly significant differences for the majority of the characteristics, during the two successive years of study. Many correlations were noted between traits related to vegetative development and those related to flowering and reproduction. The results highlighted the important role of the provenance environmental factors, such as, the rainfall, the temperature and the altitude, respectively, on the behaviour and morphology of the local populations. Indeed, the results showed that the populations (Aoulef, Tamentit, In Ghar and El-menea) coming from the less watered regions, showed a better performance thanks to their earliness and especially their capacity to provide a high seed yield.

As alfalfa is a perennial species, it would be interesting to continue this study over several years and several sites, and thus to better understand the mechanisms of their evolution and behaviour for planning future breeding programmes. The preservation of local populations of perennial alfalfa (*Medicago sativa*) has become more than necessary in order to valorize them. This study is a contribution to the development of fodder and livestock in Algeria and, consequently, to the improvement of the living conditions of rural populations.

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