

ANALYSIS OF BEHAVIOUR AND CHEMICAL COMPOSITION WITHIN SEVERAL LOCAL POPULATIONS OF *MEDICAGO SATIVA* L. (FABACEAE) IN HYPER-ARID REGION OF SOUTH-WESTERN ALGERIA

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Abstract

As part of the assessment and valorisation of plant genetic resources of fodder and pastoral interest in Algeria, a study of the behaviour and chemical composition of seven local populations of *Medicago sativa* L. (El-menea, Tamentit, Temacine, Timimoun, El Guerrara, Aoulef, In Ghar) was carried out at the INRAA station in Adrar (hyper-arid region). The experimental design was a randomized complete block design with three replications. Sixteen variables relating to the growth and chemical composition of the populations were taken into account (seedling emergence date (SE), maximum height of plant (MH), beginning date of flowering stage (BF), weight of green matter (GM), weight of dry matter (DM), mineral matter (MM), organic matter (OM), crude cellulose (CC), total nitrogenous matter (TNM), phosphorus (P), calcium (Ca), acid detergent fibre (ADF), neutral detergent fibre (NDF), lignin (ADL), true cellulose (TC) and hemicelluloses (HC)). Three ecological factors (altitude, rainfall, temperature), characterising the different provenance regions of the populations, were also taken into account. Analysis of variance identified six significant variables (SE, BF, MM, ADL, OM and P). The correlation matrix highlighted numerous relationships among the variables. The ecological factors of the populations' origin environment, considered either separately or together, appear to influence certain parameters (BF, GM, DM, P). The altitude would have a highly significant influence on the plant's phosphorus (P) content (negative correlation), making it the only ecological factor influencing the chemical composition of local populations of perennial alfalfa. Principal component analysis identified three population groups, thereby broadening the range of options for underutilised or little-known local populations. This will encourage farmers and livestock breeders to increase the multiplication of local seeds, thereby boosting fodder production and livestock farming, and consequently helping to improve the standard of living in rural communities, particularly in Saharan areas.

Key words: Behaviour; Chemical composition; Hyper-aridity; *Medicago sativa* L.; Phytogenetic resources

Introduction

After Poaceae, the Fabaceae, commonly known as legumes, constitute the largest family of cultivated plants in the tropical and temperate regions of the world, comprising some 700 genera and 18,000 species (Langer & Hill, 1991). The Anon., (2016) signalizes 700 genera and more than 20,000 species in the Fabaceae. More recently, a new classification of the legume family, based on phylogenetic analyses, recognized six robustly supported monophyletic subfamilies (Anon., 2017).

Medicago sativa L. (Alfalfa) is reported to be one of the species belonging to the genus *Medicago* (Fabaceae) (Quezel & Santa, 1962; Issolah & Beloued, 2013; Dobignard & Chatelain, 2012). This species (*Medicago sativa*), regarded as the most important of the Mediterranean forage plants, is grown under irrigation for hay, and only the wild, natural forms are suitable for grazing in difficult areas (Ben Chaâbane & Delgado, 1995).

Because of the highly heterogeneous nature of alfalfa and its high level of outcrossing, selection pressures could lead to significant genetic shifts resulting in populations that are more adaptive to their surroundings than the original synthetic and this could contribute to ferality potential (Bagavathiannan & Van Acker, 2009).

According to You *et al.*, (2022), Alfalfa (*Medicago sativa*) is high-quality forage due to its high protein and mineral contents and good palatability. Desta (2026) indicates that *Medicago sativa* L. is a multifunctional perennial forage legume that delivers significant agronomic, nutritional, and ecological benefits within integrated crop-livestock system. In Algeria, alfalfa is the main forage crop grown in the Saharan regions (Chaabena et Abdelguerfi, 2001). In this country, this plant (*Medicago sativa* L.) is of great interest in oasis agricultural systems (Chabouni *et al.*, 2019). Unfortunately, the areas reserved for local populations of perennial alfalfa (*Medicago sativa* L.), in Algerian Saharian oases, have declined significantly,

in favor of other introduced cultivars ; their preservation has become more than necessary (Chabouni *et al.*, 2024). Furthermore, the studies conducted on the chemical composition and nutritional value of Algerian populations of perennial alfalfa are scarce. Some local populations have been little studied, whilst others are generally little known. Consequently, certain populations are under-utilised or neglected by farmers.

For these reasons, studies are conducted to valorize the local populations of *Medicago sativa*. Currently, there is considerable interest in the relationships between morphological growth characteristics and chemical composition on one hand, and on the other, the ecological factors of the populations origin environment in order to know more about the mechanism of their evolution for better use. The present work forms part of the assessment and valorisation of plant genetic resources of forage and pastoral interest in Algeria and follows on from numerous studies conducted on wild and local fodder legumes (Issolah *et al.*, 2014a; Issolah *et al.*, 2014b; Issolah *et al.*, 2016; Chabouni *et al.*, 2019; Chabouni *et al.*, 2024; Issolah *et al.*, 2024; Issolah *et al.*, 2025).

Material and Methods

Following surveys carried out by INRAA (National Institute of Agronomic Research of Algeria), among farmers throughout south-western Algeria (Chabouni *et al.*, 2024), seven local populations of alfalfa (*Medicago sativa* ; Fabaceae), coming from different eco-geographical regions (Table 1), were the subject of an experimental trial (field) at the INRAA Adrar station (hyper-arid climate). Then, several analyses were made at the laboratory. Overall, this experiment consisted in evaluating several parameters (16) linked to the vegetative development, flowering and chemical composition of the different populations.

Table 1. Presentation of algerian populations of *Medicago sativa* L. provenances and climates

Population name	provenance area	Climate
El-menea	Ghardaia	Arid
Tamentit	Touat, Adrar	Hyper-arid
Temacine	Touggourt, Ouargla	Arid
Timimoun	Gourara, Timimoun	Hyper-arid
El-Guerrara	Ghardaia	Arid
Aoulef	Tidikelt, Adrar	Hyper-arid
In Ghar	In Salah	Hyper-arid

Source: Chabouni *et al.*, 2024

The experimental design was a randomized complete block design with three replications. For each population, 400 seeds m⁻² (4 lines with 100 seeds per line) were sown (09 February 2025). The soil texture was sandy loam and the pH was alkaline (7.6). The electrical conductivity was 5.92. The total limestone (CaCO₃) was 8.31%. The annual rainfall was 7.37 mm (2024/2025) and the average temperatures of the year were 34.08°C (maximum mean) and 18.66°C (minimum mean), respectively. The cutting stage considered for all populations was at the beginning of flowering stage (BF). The trial was carried out under localised irrigation (drip). The irrigation conditions were identical for the three (03) blocks.

After each mowing, the plant material was dried to constant weight at 65°C in a ventilated oven. After grinding, a second drying was carried out to determine the residual dry matter at a temperature of 103°C (Anon., 1982).

The parameters (16) considered during the experiment were as follows : seedling emergence date (SE expressed in number of days after sowing), maximum height of plant (MH, cm / mean of three randomly chosen plants per square meter), beginning date of flowering stage (BF expressed in number of days after emerging), weight of green matter (GM, g/m²), weight of dry matter (DM, g/m²), mineral matter (MM in % DM) (Anon., 1977), organic matter (OM in % DM), deduced by the equation (DM-MM), crude cellulose (CC in % DM) determined by the Weend method (Ampureo, 2008), total nitrogenous matter (TNM in % DM) determined by the Kjeldhal method (international reference method), phosphorus (P in % DM) determined by spectrophotometry, calcium (Ca in % DM) determined by titrimetric method (standardised analytical technique), acid detergent fibre (ADF in % DM), neutral detergent fibre (NDF in % DM), lignin (ADL in % DM), true cellulose (TC in % DM) (Van Soest *et al.*, 1991), hemicellulose content (HC in % DM) deduced by the equation (NDF-ADF) (Zarovali *et al.*, 2007). All results relating to chemical composition are expressed as a percentage of dry matter (DM) (Anon., 2009).

Three (03) supplementary variables (altitude, rainfall, temperature), related to the ecological conditions of the provenance regions of populations, were also taken into account.

The data obtained were subjected to an analysis of variance supported by the Tukey Test (homogeneous groups), an analysis of the correlation matrix and a principal component analysis (PCA) of all the variables and populations. Statistical processing was carried out using Minitab (2016) and XLSTAT (2016) softwares.

Results and Discussion

Variance analysis: Overall, the analysis of variance identified six (06) significant variables (SE, BF, MM, ADL, OM and P) out of a total of sixteen (16) studied variables (Table 2). Among the significant variables, two are related to the plant's behaviour during the vegetative stage (SE) and flowering stage (BF), whilst four variables (MM, ADL, OM, P) are directly linked to the chemical composition of local alfalfa populations.

Thus, depending on the probability, the results of the variance analysis indicated that only one variable (BF/Beginning of flowering) was found to be very highly significant. Two variables (ADL/Lignin, OM/Organic matter) were found to be highly significant, whereas three variables (SE/Seedling emergence date, MM/Mineral matter, P / Phosphorus) were found to be significant (Table 2).

Using Tukey's test, the analysis of variance identified distinct homogeneous groups within significant variables (SE, BF, MM, ADL, OM, P). Thus, the emergence date (SE) revealed a single group of populations ranging from 6 to 10.33 days after sowing, in Tamentit and Temacine populations, respectively (Table 3). At the beginning of flowering stage (BF), three population groups were identified. Group 'a' comprised the latest-flowering population (El-Guerrara) (89.67 days), whilst group 'c' comprised the earliest-flowering populations (Aoulef, In Ghar), at 78.33 and 81.33 days after emergence, respectively. The remaining populations showed intermediate values (Table 3).

Table 2. Variance analysis linked to the growth and chemical composition within seven local populations of *Medicago sativa* L. in Algeria.

Characteristics	Min	Max	Mean	S.D	FObs	Probability
SE (days)	6.00	10.33	7.86	2.06	3.92	0.021*
BF (days)	78.33	89.67	84.43	4.35	12.43	0.000***
GM (g/m ²)	564	1079	757.30	279.70	1.34	0.313 ^{NS}
DM (g/m ²)	136.6	238.8	184.40	66.30	2.35	0.097 ^{NS}
MH (cm)	46.11	52.44	48.45	4.65	1.08	0.426 ^{NS}
MM % DM	14.12	16.75	15.27	1.37	4.39	0.014*
TNM % DM	16.71	19.31	18.10	2.21	0.51	0.792 ^{NS}
CC % DM	21.93	25.17	23.78	1.62	1.95	0.152 ^{NS}
NDF % DM	32.69	37.76	35.18	3.11	1.04	0.447 ^{NS}
ADF % DM	23.68	28.04	25.54	2.86	1.02	0.457 ^{NS}
HC % DM	8.32	9.84	9.15	0.80	1.14	0.396 ^{NS}
TC % DM	7.18	16.53	12.72	4.64	1.76	0.190 ^{NS}
ADL % DM	6.32	18.51	10.57	5.12	7.64	0.002**
OM % DM	76.56	73.62	75.18	1.53	5.71	0.005**
Ca % DM	1.94	2.64	2.27	0.34	2.87	0.057 ^{NS}
P % DM	0.33	0.47	0.40	0.06	3.01	0.049*

Minimum: Mean of population; Maximum: Mean of population; Mean: Mean of the species; SE: Seedling emergence date; BF: Beginning date of flowering stage; GM: Weight of green matter; DM: Weight of dry matter; MH: Mean of three randomly chosen plants per square meter; MM: Mineral matter; OM: Organic matter; TNM: Total nitrogenous matter; CC: Crude cellulose; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; HC: Hemicellulose; TC: True cellulose; ADL: Lignin; Ca: Calcium; P: Phosphorus. SD: Standard deviation; Fobs: Observed F; Probability: *p<0.05; **p<0.01 and ***p<0.001

In the case of mineral matter (MM), the analysis of variance revealed two population groups. Group 'a' comprised El-menea population (16.75% DM), whilst group 'b' comprised Temacine and Timimoun populations, with 14.12% and 14.20% DM, respectively (Table 3). With regard to lignin (ADL), which is a key indicator of nutritional value, the analysis of variance identified three population groups. Group 'a' comprised the population (Timimoun), having the highest lignin content (18.51% DM), whilst group 'c' comprised the populations (Tamentit, Inghar) with the lowest contents, at 6.32% DM and 6.92% DM respectively. The other populations showed intermediate values (Table 3). In the case of organic matter (OM), analysis of variance revealed two population groups. Group 'a' consisted of the populations (Timimoun, Temacine), which were the richest with 76.56% DM and 76.52% DM, respectively, whilst group 'b' comprised the population (El-Menea), the poorest in this respect (73.62% DM). The other populations fell between these extremes (Table 3).

With regard to phosphorus (P), the analysis of variance identified two population groups. Group 'a' consisted of the population (Temacine) with the highest phosphorus content (0.47% DM), whilst group 'b' included the population (El-Menea) with the lowest value (0.33% DM). The remaining populations fell between these two extremes (Table 3).

An earlier study conducted by Rahal-Bouziane (2005) on Algerian populations of perennial alfalfa, originating from the Hoggar region (southern Algeria) showed that, at the full-flowering stage, yields and dry matter content were better than at the early-flowering stage, however, the forage was of lower quality.

Alane *et al.*, (2024) demonstrated that the results of variance analysis, applied to the chemical parameters of two Algerian cultivars (Tamentit and El menea) and two foreign varieties (Mamantane and Triade), indicated a highly significant difference for mineral matter and a very highly

significant difference for crude fibre, at the flower bud stage. These authors also reported a very highly significant difference for all chemical parameters, at the beginning of flowering stage and highlighted the importance of this stage for selecting the best cultivars (Tamentit and El menea), which have a high nitrogen content and a digestibility of over 60% for the two studied stages (flower bud and early flowering) (Alane *et al.*, 2024).

Under different ecological conditions (Romania), Vintu *et al.*, (2012) recommend the harvesting of alfalfa at the beginning of bud-formation period, regardless the fertilization level, to ensure a good quality of alfalfa forage.

Giger (1985) opines that lignin is one of the mean factors determining the energy value of feedstuffs; being indigestible, it reduces the digestibility of the other cellwall components and is therefore important in the prediction of the dietary energy content. Baumont *et al.*, (2016) noted that legumes were richer in total nitrogen (TN) than grasses, as well as in certain minerals, particularly calcium. However, due to lower NDF digestibility, the digestibility of organic matter (dOM) in stemmed legumes (alfalfa, red clover, sainfoin), and consequently their energy value, is generally lower than or equivalent to that of grasses (Baumont *et al.*, 2016).

Correlation matrix: Through the present study, the correlation matrix revealed numerous relationships among the variables (Table 4).

Populations characterised by an early emergence date, also flower early, have a high dry matter content, a high mineral content, a low total nitrogen content, a low organic matter content and, finally, a high calcium content.

Populations characterised by a high green matter weight also have a high dry matter weight and a low hemicellulose content. Populations with a high NDF content are characterised by high ADF and ADL contents and a low true cellulose (TC) content.

Table 3. Homogenous groups (Test of Tukey) within seven local populations of *Medicago sativa* L. in Algeria.

Variables populations	SE (days)	BF (days)	GM (g/m ²)	DM (g/m ²)	MH (cm)	MM % DM	TNM % DM	CC % DM	NDF % DM	ADF % DM	HC % DM	TC % DM	ADL % DM	OM % DM	Ca % DM	P % DM
El-Menea	6.33 ^a	83.33 ^{bc}	708.00	193.80	48.33	16.75 ^a	16.71	25.00	35.27	25.81	9.16	13.20	11.57 ^{abc}	73.62 ^b	2.30	0.33 ^b
Aoulef	6.67 ^a	78.33 ^c	737.00	182.80	46.22	16.13 ^{ab}	17.99	24.19	37.76	28.04	9.20	9.27	15.50 ^{ab}	74.33 ^{ab}	2.47	0.41 ^{ab}
Tamentit	6.00 ^a	83.33 ^{bc}	807.30	232.70	46.11	15.92 ^{ab}	17.44	23.23	33.92	24.12	9.45	16.53	6.32 ^c	74.47 ^{ab}	2.65	0.42 ^{ab}
El-Guerrara	10.00 ^a	89.67 ^a	593.30	136.60	46.56	14.29 ^{ab}	19.31	25.17	33.92	24.26	9.02	15.14	7.21 ^{bc}	76.02 ^{ab}	1.94	0.35 ^{ab}
In-Ghar	7.33 ^a	81.33 ^c	1079.0	238.80	50.33	15.49 ^{ab}	18.58	21.93	32.69	23.68	8.32	14.62	6.92 ^c	74.74 ^{ab}	2.33	0.38 ^{ab}
Timimoun	8.33 ^a	87.67 ^{ab}	813.00	167.90	52.44	14.20 ^b	18.14	23.71	37.15	27.54	9.10	7.18	18.51 ^a	76.56 ^a	1.94	0.42 ^{ab}
Temacine	10.33 ^a	87.33 ^{ab}	564.00	138.00	49.17	14.12 ^b	18.57	23.23	35.55	25.32	9.84	13.12	7.98 ^{bc}	76.52 ^a	2.25	0.47 ^a

SE: Seedling emergence date; BF: Beginning date of flowering stage; GM: Weight of green matter; DM: Weight of dry matter; MH: Mean of three randomly chosen plants per square meter; MM: Mineral matter; OM: Organic matter; TNM: Total nitrogenous matter; CC: Crude cellulose; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; HC: Hemicellulose; TC: True cellulose; ADL: Lignin; Ca: Calcium; P: Phosphorus. Letters (a; b; c): Homogeneous groups

With regard to the relationships among the various variables and the ecological factors of provenance regions, the correlation matrix indicated that populations which flower early (BF) came from areas with high temperatures. Populations characterised by high green matter weight came from regions with high temperatures and low rainfall. Populations characterised by high dry matter weight came from regions with low rainfall. Populations characterised by high phosphorus content came from low-altitude regions (Tables 4 and 5).

Thus, temperature appears to be the only environmental factor influencing the flowering of local populations of perennial alfalfa. Rainfall and temperature are the two ecological factors that simultaneously affect the plant's green matter weight. Rainfall alone affects the plant's dry matter weight whereas altitude appears to have no effect on the plant's vegetative growth and the beginning of flowering ; however, it would have a highly significant influence on the plant's phosphorus content, making it the only ecological factor (altitude) influencing the chemical composition of local populations of perennial alfalfa.

Table 5. Relationships of some characteristics linked to growth and chemical composition with the ecological factors (altitude, rainfall, temperature) of the provenance regions within seven (07) local populations of *Medicago sativa* L. in Algeria.

Characteristics	Ecological factors of the provenance regions of populations		
	Altitude	Rainfall	Temperature
Beginning of flowering (BF)	-0.09	0.74	-0.76
	0.84 NS	0.06 NS	0.05*
Green matter (GM)	0.13	-0.81	0.78
	0.78 NS	0.03*	0.04*
Dry matter (DM)	0.14	-0.78	0.73
	0.76 NS	0.04*	0.06 NS
Phosphorus (P)	-0.90	- 0.22	0.18
	0.01**	0.64 NS	0.69 NS

Cell content: Coefficient of pearson and probability (*p≤0.05; **p≤0.01); NS: No significant

Principal component analysis: To better illustrate the results obtained in this study, a principal component analysis was performed using the most significant variables (Fig. 1).

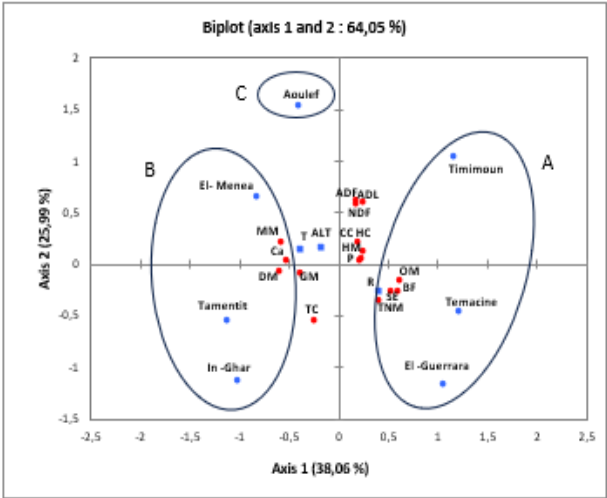


Fig. 1. Principal Component analysis of characteristics linked to the growth and chemical composition within seven local populations of *Medicago sativa* L. in Algeria.

Table 4. Correlation matrix of characteristics linked to growth and chemical composition within eight local populations of *Medicago sativa* L. in Algeria.

Characteristics	SE	BF	GM	DM	MH	MM	TNM	CC	NDF	ADF	HC	TC	ADL	OM	Ca	P	T	R
BF	0.78																	
GM	0.04	-0.51																
DM	0.21	0.24	0.84															
MH	-0.83	-0.67	0.02	-0.03														
MM	0.23	0.27	0.38	0.95	-0.42													
TNM	0.62	0.55	0.40	0.95	0.34	-0.78												
CC	-0.90	0.03	0.50	0.10	0.09	0.04	-0.15											
NDF	0.10	0.30	-0.71	-0.56	-0.41	0.10	0.75	-0.22	0.35									
ADF	0.82	0.52	0.08	0.19	0.37	0.83	0.75	-0.22	0.35	0.64	0.43							
HC	-0.02	-0.13	-0.36	-0.42	0.12	-0.03	0.95	-0.25	0.33	0.98	0.98	0.01						
TC	0.96	0.77	0.43	0.35	0.81	0.95	0.64	0.43	0.98	0.98	0.98	0.01	0.00					
ADL	-0.12	-0.22	-0.21	-0.32	0.18	0.05	-0.25	0.33	0.98	0.98	0.98	0.01	0.00	0.00				
OM	0.80	0.63	0.65	0.49	0.70	0.91	0.59	0.47	0.98	0.98	0.98	0.01	0.00	0.00	0.00			
Ca	0.25	0.28	-0.78	-0.49	-0.28	-0.18	-0.19	0.27	0.42	0.24	0.26	-0.27	0.1	0.82	0.82	0.18		
P	0.59	0.54	0.04	0.27	0.54	0.69	0.68	0.55	0.35	0.60	0.91	0.57	0.36	-0.30	-0.69	0.50	0.23	
T	-0.03	0.08	0.02	0.31	-0.51	0.17	0.06	-0.13	-0.88	-0.93	-0.03	0.17	0.42	0.51	0.08	0.23	0.69	
R	0.94	0.87	0.97	0.50	0.25	0.71	0.90	0.78	0.01	0.00	0.94	0.01	0.42	0.51	0.08	0.23	0.69	
ALT	-0.15	-0.13	-0.01	-0.21	0.41	0.00	-0.24	0.27	0.88	0.94	0.01	-0.97	0.05	0.05	0.23	0.69	0.93	
	0.75	0.79	0.98	0.64	0.36	1.00	0.60	0.57	0.01	0.00	0.98	0.00	0.26	0.91	0.25	0.62	0.93	
	0.87	0.78	-0.33	-0.68	0.47	-0.99	0.71	-0.09	0.14	0.06	0.26	-0.27	0.1	0.82	0.82	0.18	0.93	
	0.01	0.04	0.46	0.09	0.29	<0.0001	0.08	0.85	0.76	0.91	0.57	0.55	0.36	-0.30	-0.69	0.50	0.93	
	-0.71	-0.76	0.27	0.68	-0.52	0.71	-0.54	-0.35	-0.12	-0.14	0.17	0.36	-0.30	-0.69	0.50	0.93	0.93	
	0.08	0.05	0.56	0.09	0.23	0.08	0.21	0.44	0.80	0.77	0.71	0.42	0.51	0.08	0.23	0.69	0.93	
	0.26	0.04	-0.10	-0.12	0.18	-0.44	0.18	-0.54	0.30	0.18	0.56	-0.21	0.05	0.50	0.23	0.69	0.93	
	0.57	0.94	0.84	0.79	0.70	0.33	0.69	0.21	0.52	0.70	0.19	0.65	0.91	0.25	0.62	0.93	0.93	
	-0.63	-0.76	0.78	0.73	0.01	0.38	-0.11	-0.58	0.05	0.15	-0.47	-0.19	0.19	-0.37	0.52	0.93	0.93	
	0.13	0.05	0.04	0.06	0.98	0.40	0.82	0.17	0.92	0.75	0.28	0.69	0.68	0.41	0.23	0.69	0.93	
	0.73	0.74	-0.81	-0.78	-0.21	-0.45	0.37	0.60	-0.14	-0.25	0.38	0.30	-0.33	0.41	-0.53	-0.22	0.93	
	0.06	0.06	0.03	0.04	0.65	0.32	0.42	0.15	0.76	0.59	0.39	0.52	0.47	0.37	0.22	0.64	0.93	
	-0.41	-0.09	0.13	0.14	-0.12	0.45	-0.30	0.61	0.01	0.14	-0.50	-0.09	0.29	-0.47	-0.24	-0.90	-0.001	
	0.36	0.84	0.78	0.76	0.79	0.31	0.51	0.15	0.98	0.76	0.25	0.84	0.52	0.29	0.61	0.01	0.99	

Cell content: Pearson coefficient and Probability (*p≤0.05; **p≤0.01; ***p≤0.001). SE: Seedling emergence date; BF: Beginning date of flowering stage; GM: Weight of green matter; DM: Weight of dry matter; MH: Mean of three randomly chosen plants per square meter; MM: Mineral matter; OM: Organic matter; TNM: Total nitrogenous matter; CC: Crude cellulose; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; HC: Hemicellulose; TC: True cellulose; ADL: Lignin; Ca: Calcium; P: Phosphorus; T: Temperature; R: Rainfall; ALT: Altitude

Axis 1-2 of the principal component analysis (PCA) explain 64.05% of the total variation. Axis 1 (38.06%) is positively correlated with emergence date (SE), beginning of flowering (BF), total nitrogen (TNM) and organic matter (OM), and negatively correlated with dry matter (DM), mineral matter (MM) and calcium (Ca). Axis 2 explains 25.99% of the total variation. It is positively correlated with neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (ADL), and negatively correlated with true cellulose (TC).

Axis 1 presents the populations of Group A (Timimoune, Temacine, El Guerrara), characterised by late emergence (SE), a similarly late beginning of flowering (BF), and high levels of total nitrogen (TNM) and organic matter (OM). In contrast, the populations in Group B (El-Menea, Tamentit and In Ghar) are distinguished by high values for dry matter (DM), mineral matter (MM) and calcium (Ca).

Axis 2 is positively correlated with Group C, comprising a single population (Aoulef), which stood out clearly for its high values of neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (ADL), as well as a low true cellulose (TC) content.

Principal component analysis thus enabled the identification of three groups (A, B, C) of local populations (*Medicago sativa*), based on various selection criteria (emergence, flowering, chemical composition).

The results of a previous study, conducted on eight genotypes of perennial alfalfa, from different origins (Tunisia, Algeria, Morocco, Italy, Iran, Australia), indicated that improving the biomass yield of alfalfa germplasm must be achieved without affecting other components of agronomic value, which are mainly : lifespan (3 to 6 years) and ease of establishment, flexibility of use (grazing, hay, silage, etc.), yield distribution throughout the year, palatability and flexibility of leaves and stems, protein content, seed production, drought tolerance, and disease resistance (Tlahig *et al.*, 2022). In a review summarising the main findings from France and Europe on the chemical composition, digestibility and palatability to sheep of the main forage species of grasses and legumes, grown as green forage, Demarquilly & Andrieu (1992) note that, at a given stage of growth, there are differences between species, but the year and location of the study have no significant influence, particularly on digestibility.

While examining the performance of 11 populations and varieties of perennial alfalfa conducted in the Ouargla region (Southern Algeria), Chaabena & Abdelguerfi (2021) reported that farmers had developed populations that were able to match, and in some cases significantly exceeded, the introduced varieties for certain traits. Alane *et al.*, (2024) reported that the stage of cutting was the main factor influencing variation in all the chemical parameters studied in Algerian and foreign cultivars of *Medicago sativa* L. (total nitrogen, mineral matter, crude fibre). Furthermore, Chabouni *et al.*, (2024) highlighted the important role of provenance environmental factors, such as, the rainfall, the temperature and the altitude, respectively, on the morphology of seven algerian populations of perennial alfalfa (El-menea, Tamentit, Temacine, Timimoun, El Guerrara, Aoulef, In Ghar). Indeed, the results showed that the populations (Aoulef,

Tamentit, In Ghar and El-menea) coming from the less watered regions, performed better thanks to their earliness and especially their capacity to provide a high seed yield (Chabouni *et al.*, 2024).

Within the same family (*Fabaceae*), previous studies (Issolah *et al.*, 2014a; Issolah *et al.*, 2014b; Issolah *et al.*, 2016; Issolah *et al.*, 2024) highlighted the relationships between certain ecological factors, characterising the natural habitats of various forage legumes in Algeria, and parameters related to the chemical composition of the plants; these studies have demonstrated the impact of one or two ecological factors (altitude, rainfall) on the chemical composition of wild or local populations within certain species (*Vicia sativa*, *Vicia narbonensis*), whilst other species (*Hedysarum coronarium*, *Trifolium subterraneum*) do not seem to be influenced by these factors.

However, the morphological characteristics, linked to various legumes (several species of vetch, several species of clovers, sulla, peas, perennial alfalfa) appear to be influenced to a greater or lesser extent by at least one ecological factor (altitude, rainfall, temperature), characterising the natural habitats and/or areas of provenance of Algerian populations (Issolah, 2006; Issolah & Abdelguerfi, 2003; Issolah, 2018; Chabouni *et al.*, 2024; Issolah *et al.*, 2025).

Analyses of ecological factors belonging to the provenance areas and their impact on the behaviour and chemical composition of forage legumes have proven essential in a plant breeding programme in order to select the most high-performing plant material for specific regions, thereby increasing the plant's chances of success and adaptation to its environment and thus meeting users' expectations and needs.

In this context, the selection of suitable and appropriate plant material could have significant socio-economic benefits, particularly in remote and disadvantaged regions, where climatic conditions are extremely harsh.

Conclusion

This study has highlighted a number of findings regarding variation in variables relating to the behaviour (vegetative development, flowering) and chemical composition of several local populations (El-Menea, Tamentit, Temacine, Timimoun, El Guerrara, Aoulef, In Ghar) of perennial alfalfa in Algeria.

It is emphasised that the variation in flowering time is highly significant in certain early local populations (Aoulef, In Ghar) compared with others, which flower relatively later (El-Guerrara). The chemical analysis also highlighted the important variation of lignin content (ADL) (highly significant probability). This parameter (ADL) is an important indicator of nutritional value (animal feed).

The correlation matrix revealed numerous relationships among the various parameters and highlighted the links between certain variables and the ecological factors (altitude, rainfall, temperature) of the provenance areas of populations.

Principal component analysis revealed the existence of three distinct groups of local populations, thereby offering users a range of options (emergence, flowering, chemical composition).

This work has enabled the evaluation and valorisation of a wide range of Algerian populations, some of which are relatively unknown and/or under-utilised, and are likely at risk of genetic erosion (Timimoun, El Guerrara, Aoulef, In Ghar). Moreover, this contribution is intended for agricultural technicians, as well as farmers and livestock breeders, with a view to encouraging the cultivation of local populations of perennial alfalfa, particularly in the extremely harsh environmental conditions of Saharan regions (hyper-arid and arid climates). – Conclusion is too long. It is even larger than the abstract. Shorten the conclusion

Acknowledgment

This work was conducted with the financial support of the Ministry of Higher Education and Scientific Research (MESRS / PNR 2021 Project).

Conflict of Interest: The authors declare that there is no conflict of interest.

Author's Contributions: **R.I:** Conceptualization, data, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, visualization, analyses, writing. **A.C:** Resources, investigation, data. **F.A and M.S.C:** Methodology, investigation, data. **Z.B. and A.T:** Methodology, formal analyses. **Z.A:** Investigation.

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