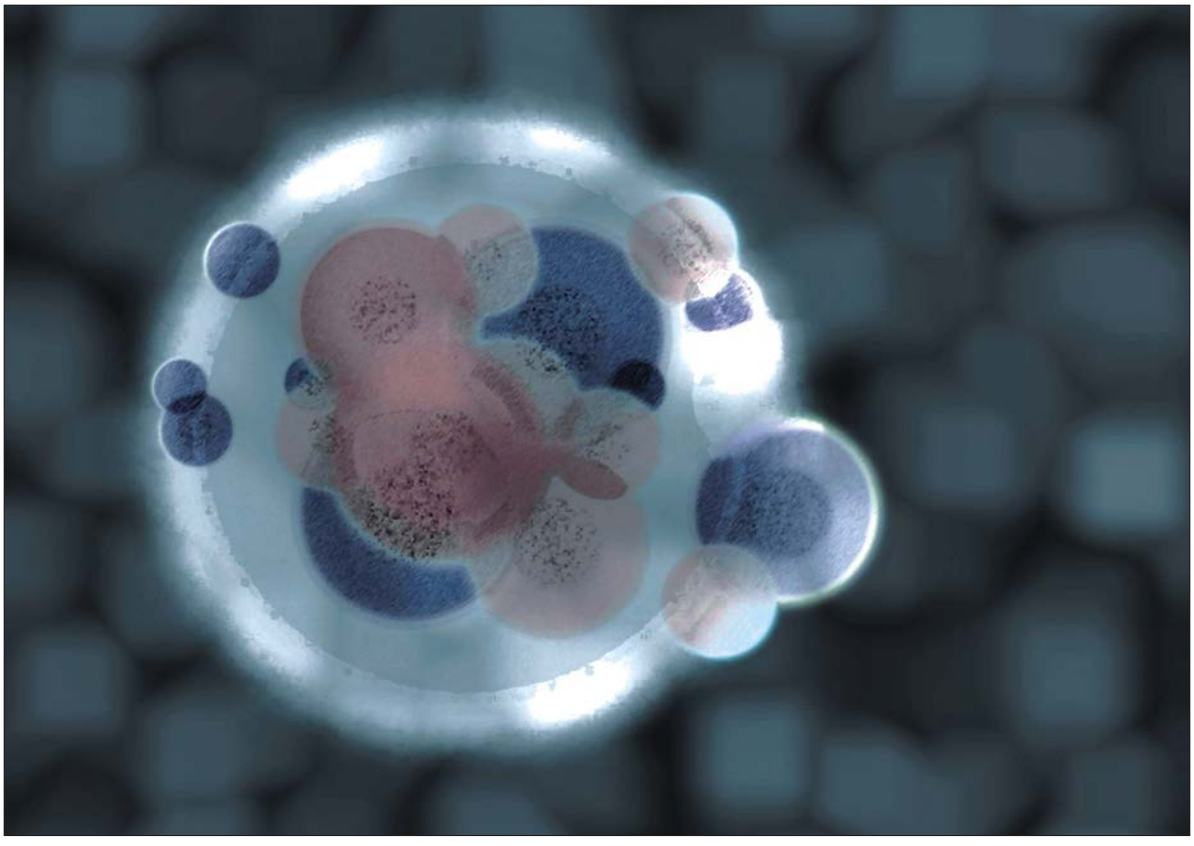

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Research Article
Analysis of the Behaviour and the Chemical Composition Within Algerian Populations of *Trifolium subterraneum* L.

[1]R. Issolah, [2]A. Tahar, [3]S. Sadi, [3]M. Adjabi, [4]F. Alane, [5]Y. Chellig-Siziani and [5]M. Lebied

[1]Division des Ressources Phytogénétiques, INRAA, CRP Mehdi Boualem, BP 37 Baraki, Alger, Algérie
[2]Département de Biologie, Université Badji Mokhtar, Annaba, Algérie
[3]Laboratoire Central, ITELV, Baba Ali, Algérie
[4]Division de Zootechnie, INRAA, BP 37 Baraki, Alger, Algérie
[5]Département des Ruminants, ITELV, Service Bovins, Baba Ali, Algérie

Abstract

Background and Objective: As part of the valorization of plant genetic resources of fodder and pastoral interest in Algeria, ten populations of subterraneun clover (*Trifolium subterraneum* L.) were subject to an evaluation. The objective of this study is the characterization of the Algerian populations of subterraneun clover on quantitative and qualitative level. This would permit to increase the number of fodder species used in the different areas of the country for developing the livestock. **Materials and Methods:** The field trial was a randomized complete block design with four replications. Then, the chemical composition of the populations was analysed at the laboratory. In this framework, several parameters were determined (Thousand seeds weight, seedlings, height, width, flowering, cutting date, green matter, mineral matter, organic matter, crude fiber, fat, phosphorus, calcium, total nitrogen, acid detergent fiber, neutral detergent fiber, lignin and hemicellulose). Two ecological factors (Altitude and rainfall) characterizing the natural habitat of the populations were considered. The data obtained have undergone statistical treatments (ANOVA/Tukey method). Then, the correlations and the principal component analysis were used for a better interpretation of the relations established between the different populations and the variables. **Results:** The variance analysis indicated that there is some variation in three variables linked to the cutting date, the thousand seed weight and the yield of green matter. It seems that there is no link between the variables and the ecological factors. The principal component analysis indicated that the calcium and the vegetative development in width are the variables, the best represented. **Conclusion:** Overall, the variables related to the chemical composition contribute more than those related to the vegetative development (number of variables), but the probabilities corresponding to the vegetative development are the most significant. This study would contribute to select the best populations of subterraneun clover in order to introduce them in the different agro-edapho-climatic areas, specially in the Mountains of the North East of Algeria, which is the most watered zone in the country.

Key words: Fodder legume, nutrients, plant genetic resources, subterraneum clover

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Corresponding Author: R. Issolah, Division des Ressources Phytogénétiques, INRAA, CRP Mehdi Boualem, BP 37 Baraki, Alger, Algérie

Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

The genus *Trifolium* belongs to the Fabaceae family and contains about 250 species around the world[1]. All species of *Trifolium* are herbaceous perennials or annuals, frequently prostrate[2]. Algeria is a Mediterranean and North African country, characterized by 37 species of the genus *Trifolium* L., among which, *Trifolium subterraneum* L., commonly called subterraneun clover and encountred in the meadows, specially the mountainous ones[3]. Subterranean clover is a fodder legume, growing in the Mediterranean climate zone[4]. This species seems to be adapted to unsalted and calcareous soils, diverse textures and a variable pH[5]. The record of Algerian populations of subterranean clover on frankly alkaline soils appears for the first time within some subspecies and/or varieties of this species[5]. The development of the livestock depends on the quantity and quality of the forages, which constitute some natural meadows in the North East of the country. The knowledge of grazed plants in difficult environments is essential to estimate their nutritional value to establish rational methods of use of available feed resources[6]. In some areas of the East of Algeria, the feed situation is characterized by an insufficient ruminant feed supply both qualitatively and quantitatively[7]. Sub clover has high nutritive value, which promote intake and animal production[8]. Mixtures of alfafa with subterraneun clover showed higher crude protein content and were more digestible[9]. The aimed of this study is to analysed the behaviour and the chemical composition of the natural populations, coming from different natural habitats of Algeria in order to introduce them in a programme of selection for developing the forages and the livestock. It would contribute also to resolve the problem of the fodders in Algeria by increasing and diversifying the number of the species according to the different agro-edapho-climatic conditions of the country. The present study follows the different studies realized on natural legumes, in the framework of the development and the preservation of plant genetic resources of fodder and pastoral interest in Algeria[5,10-16].

MATERIALS AND METHODS

Several populations of subterraneun clover were collected by INRAA (National Institute of Agricultural Research of Algeria) in 2010, across the North East of Algeria[5]. Among these populations, ten coming from different natural habitats (Table 1) have been the subject of a field trial in order to evaluate several parameters related to the vegetative development, flowering, pods and chemical composition.

Table 1: Ecological characterization of the natural habitats of some Algerian populations of *Trifolium subterraneum* L.

No. of populations	Origin area	Altitude (m)	Rainfall (mm)
1	Guelma	170	600
2	Guelma	200	558
3	Tarf	665	661
4	Tarf	555	661
5	Souk Ahras	950	800
6	Souk Ahras	1040	700
7	Souk Ahras	810	700
8	Souk Ahras	800	900
9	Souk Ahras	1110	700
10	Skikda	110	562

Source: Issolah *et al.*[5]

For each population, 250 seeds m^{-2} (5 lines spaced by 25 cm at a rate of 50 seeds per line) were sown (1st December, 2011). A randomized complete block design was used with four replications.

The texture of the soil was silty clayey. The pH was alkaline (7.90). The annual rainfall was 818.2 mm (2011/2012) and the average temperatures of the year were 22.34°C (maxima average) and 13.83°C (minima average), respectively.

The considered parameters are the following: The thousand seed weight (TSW: g), the emergence of seedlings (SE: Number of days after the sowing), the maximum height reached by the population (HM: cm), the maximum width attained by the population (WM: cm) and the date of appearance of the first flowers (1F: Number of days after the sowing). Following the cut date (CD: Flowering-beginning of the formation of pods) for all considered populations, several analysis were performed in the laboratory through various techniques: Weight of green matter (GM: g m^{-2}), the mineral matter (MM in DM%), the organic matter (OM in DM%), the crude fiber (CF in DM%) by Weende method, the fat (F in DM%), the phosphorus (P in DM%) by spectrophotometry, the calcium (Ca in DM%) by titrimetric method[17], the total nitrogen (TN in DM%) by the Kjeldahl method, the acid fiber (ADF: Acid detergent fiber in DM%), the neutral fiber (NDF: Neutral detergent fiber in DM%) and the lignin (ADL in DM%)[18].

The hemicellulose (Hcell in DM%) was deduced by the equation (NDF-ADF)[19]. It was also deduced the green matter yield (GMY: kg ha^{-1}) for each population. The results are expressed in percentage of Dry Matter (DM%) for the various parameters related to the chemical composition. The data obtained (40 for each variable/17 variables in total) have undergone statistical treatments (ANOVA/Tukey method). The variation (statistical parameters, means of populations) was analysed within the 2 supplementary variables (TSW, GMY). Then, the correlations (matrix of correlations) between all variables and two ecological factors (altitude (ALT) and rainfall

(R)) of the natural habitats of the populations (21 variables) were realized. At the end, the Principal Component Analysis (PCA) was used for a better interpretation of the relations established between the different populations and the variables. Statistical treatments were performed using the software Minitab[20] and XLSTAT[21].

RESULTS

Variance analysis: The variance analysis (ANOVA) indicated that there is no significant variation for almost all the characteristics concerning the vegetative development, the flowering and the chemical composition of *T. subterraneum*, exception made for the cutting date (flowering-formation of the fruiting heads stage), which present a significant probability (0.046*) (Table 2). The results of statistical parameters applied on the means of populations, mentioned a variation between the populations, concerning the thousand seed weight (mean of the species: 5.56 g) and the yield of green matter (mean of the species: 20.007 t ha^{-1}) (Table 3). Previous studies conducted on several species (*T. scabrum*, *T. campestre*, *T. tomentosum*, *T. glomeratum*, *T. fragiferum*, *T. resupinatum*, *T. spumosum*, *T. striatum*, *T. bocconei*, *T. arvense*, *T. ligusticum* and *T. repens*) of the same genus, showed an interesting morphological variation between the species, particularly concerning the pods, the seeds and the fruiting heads[22,23].

Matrix of correlations: Several significant relationships have been identified through the correlation matrix (Table 4). The vegetative development in width is positively correlated with the green matter (0.006*), the crude fiber (0.015*) and the fat (0.006**). The vegetative development in height is positively correlated with the green matter (0.000***), the fat (0.037*) and the hemicellulose (0.043*). The green matter is positively correlated with fat (0.012*). The mineral matters are negatively correlated to the lignin (0.028*). The organic matter and the calcium are correlated positively with the lignin (0.028* and 0.031*, respectively). Thus, many relationships exist between the characteristics related to the vegetative development and the chemical composition of populations of subterranean clover. Overall, the probabilities corresponding to the vegetative development are the most significant.

Principal component analysis: The principal component analysis is based on a total of 18 variables. The plan 1-2 extracts 51% of information. The classification of variables, based on their contribution, permitted to establish the following descending order (Fig. 1):

Ca>WM>MM>OM>CF>GM>F>HM>ADF>ADL>Rain>
ALT>NDF>Hcell>CD>SE>TSW>1F

The calcium and the vegetative development in width are the best represented comparing to the others variables.

Table 2: Results of univariate analysis (ANOVA) of the growth and the chemical composition parameters within some Algerian populations of subterraneun clover

Parameters	MIN	MAX	MEAN	Fobs	Probability
SE (days)	32	34	32.2	1.00	0.464^{NS}
WM (cm)	62.3	88.3	70.5	1.24	0.310^{NS}
HM (cm)	7.4	10.2	8.35	0.55	0.826^{NS}
1F (days)	139	139	139	-	-
CD (days)	149	155.3	153.74	2.30	0.046*
GM (g m^{-2})	1097.3	5003.2	2000.65	1.34	0.265^{NS}
MM (DM%)	12.5	19.9	16.2	0.46	0.890^{NS}
OM (DM%)	80.1	87.5	83.8	0.46	0.890^{NS}
TN (DM%)	11.2	13.5	12.25	1.15	0.362^{NS}
CF (DM%)	15.7	20.4	18.54	1.28	0.294^{NS}
F (DM%)	1.1	2	1.44	1.07	0.415^{NS}
Ca (DM%)	0.8	1.1	1.01	1.05	0.425^{NS}
P (DM%)	Trace	Trace	Trace	-	-
ADF (DM%)	28	31.1	29.03	0.99	0.468^{NS}
NDF (DM%)	36.9	40.2	38.33	1.37	0.249^{NS}
ADL (DM%)	7.8	9.4	8.53	0.66	0.739^{NS}
Hcell (DM%)	7.6	10.7	9.29	1.25	0.306^{NS}

MIN: Minimum mean of a population, MAX: Maximum mean of a population, MEAN: Mean of the species, Signification: *p<0.05 and NS: No significant

Table 3: Variation of some yield components (statistical parameters) within some Algerian populations of subterraneun clover

Parameters	Minimum	Maximum	Mean	Standard deviation	Coefficient of variation (%)
TSW (g)	4.52	8.41	5.56	1.07	19.33
Yield (kg ha^{-1})	10973	50032	20007	11958	59.77

Minimum: Value reached by a population, Maximum: Value reached by a population, Mean: Value of the species and TSW: Thousand seed weight

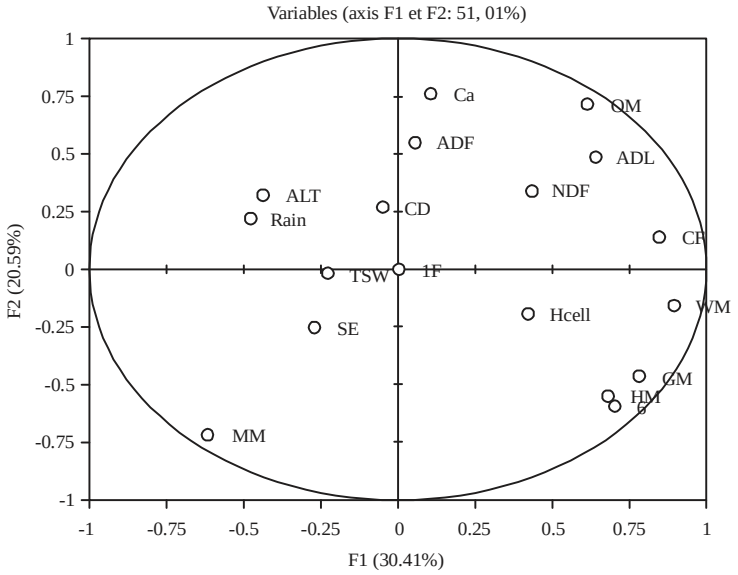

Fig. 1: Cercle of correlations (variables) of the principal component analysis wthin Algerian populations of subterraneun clover, TSW: Thousand seed Weight, SE: Emergence of seedlings, HM: Maximum height, WM: Maximum width, 1F: Date of appearance of the first inflorescences, CD: Cut date, GM: Weight of green matter, MM: Mineral matter, OM: Organic matter, CF: Crude fiber, F: Fat, Ca: Calcium, Hcell: Hemicellulose, ADF: Acid detergent fiber, NDF: Neutral detergent fiber, ADL: Lignin, ALT: Altitude and Rain: Rainfall

Table 4: Relations between the parameters linked to the growth, the chemical composition and the ecological factors of the natural habitat within some Algerian populations of subterraneun clover

Parameters	SE	WM	HM	CD	GM	MM	OM	Ca	F
HM	-0.342	0.575	-	-0.375	0.913	0.091	-0.091	-0.198	0.662
	0.333	0.082		0.286	0.000***	0.802	0.802	0.583	0.037
GM	-0.265	0.797	0.913	-0.154	-	-0.078	0.078	-0.165	0.754
	0.459	0.006**	0.000***	0.672		0.831	0.830	0.648	0.012
CF	-0.396	0.736	0.483	0.056	0.545	-0.624	0.624	0.172	0.585
	0.257	0.015*	0.157	0.877	0.103	0.054	0.054	0.635	0.075
F	0.178	0.793	0.662	-0.106	0.754	-0.092	0.092	-0.411	-
	0.622	0.006**	0.037*	0.770	0.012*	0.800	0.800	0.238	
ADL	-0.349	0.353	0.303	-0.348	0.269	-0.688	0.688	0.680	0.088
	0.323	0.318	0.395	0.324	0.452	0.028*	0.028*	0.031*	0.809
Hcell	-0.624	0.141	0.648	-0.393	0.415	0.102	-0.103	-0.011	0.206
	0.054	0.698	0.043*	0.262	0.233	0.778	0.778	0.976	0.568
R	-0.408	-0.556	-0.174	-0.304	-0.291	0.318	-0.317	0.493	-0.603
	0.242	0.095	0.630	0.394	0.415	0.371	0.371	0.148	0.065
ALT	-0.504	-0.486	-0.258	0.071	-0.403	0.119	-0.119	0.306	-0.600
	0.138	0.155	0.471	0.845	0.248	0.743	0.743	0.391	0.067

Content of cell: Pearson correlation, Probability (signification): *p<0.05, **p<0.01 and ***p<0.001, SE: Emergence of seedlings, HM: Maximum height, WM: Maximum width, CD: Cut date, GM: Weight of green matter, MM: Mineral matter, OM: Organic matter, CF: Crude fiber, F: Fat, Ca: Calcium, Hcell: Hemicellulose, ADL: Lignin, ALT: Altitude and R: Rainfall

Overall, the variables related to the chemical composition contribute more than those related to the vegetative development, while the variables linked to the ecological factors occupy an intermediate position.

The distribution of variables and populations (Fig. 2) indicated that the axis 1 is the axis of architecture (WM, HM and GM) and chemical composition (CF, F and ADL) of the plants. Axis 2 is the axis of reproduction (1F) and the mineral and organic elements (MM, OM and Ca).

Thus, the axis 1 is positively represented mainly by 6 variables (WM, HM, GM, CF, F and ADL) and a group A of two populations (2, 3). Negatively, it is represented by a group B of 5 populations (4, 5, 8, 9 and 10). The populations of the group A are characterized by relatively high values (Fig. 2). The

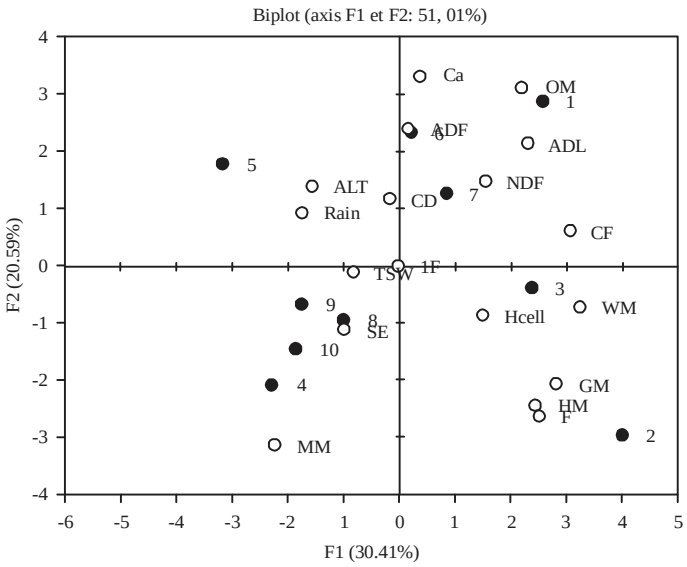

Fig. 2: Dispersion of individuals (populations) and variables of the principal component analysis within Algerian populations of subterraneun clover. Group A: Populations 2, 3, Group B: Populations 4, 5, 8, 9, 10 and Group C: Populations 1, 6, 7, TSW: Thousand seed Weight, SE : Emergence of seedlings, HM: Maximum height, WM: Maximum width, 1F: Date of appearance of the first inflorescences, CD: Cut date, GM: Weight of green matter, MM: Mineral matter, OM: Organic matter, CF: Crude fiber, F: Fat, Ca: Calcium, Hcell: Hemicellulose, ADF: Acid detergent fiber, NDF: Neutral detergent fiber and ADL: Lignin, ALT: Altitude and Rain: Rainfall

axis 2 is positively represented mainly by two variables (OM, Ca) and a group C of three populations (1, 6 and 7), which are characterized by high values (Fig. 2).

DISCUSSION

Several results were obtained through the present study. A variation was indicated through three variables linked to the morphological aspects (cutting date, thousand seed weight, yield). Many relationships were also established between different variables corresponding to the vegetative development and the chemical composition of the Algerian populations of subterraneun clover.

In Northern Greece, Kyriazopoulos *et al.*[24] obtained as mean values, 15% for crude protein, 45% for NDF, 38% for ADF and 6% for ADL in *Trifolium subterraneum* cv. M. baker (T. sub). In spain, Pereira-Crespo *et al.*[25] noted within some species of the genus *Trifolium*, across harvest dates that mean values are ranging from 17-19.1% for crude protein and from 33.6-35.6% for NDF.

With 11.2-13.5% for the total nitrogen and 36.9-40.2% for NDF, the obtained results indicated lower values for the first parameter (N) and intermediate for the second one (NDF), comparing to those obtained by some authors in Mediterranean countries[24,25].

According to Ridley *et al.*[26], there was a significant relationship between acid detergent fibre content and organic matter, whereas it seems that there is no significant link between ADF and OM through the present study.

The results signalized also that there is no link between the variables linked to the morphological and chemical aspects (vegetative development, flowering, chemical composition) with the factors of the natural habitats (Altitude and rainfall) of the Algerian populations of subterraneun clover. The same results were obtained in some Algerian populations of *Sulla coronaria* (L.) Medik, Syn, *Hedysarum coronarium* L.[15].

Previous studies indicated that several relationships were established between the morphological characteristics (vegetative development, flowering, formation of fruiting heads) and the ecological factors (Altitude and rainfall) of the natural habitats within the algerian populations of several species corresponding to the genera *Trifolium* L. and *Hedysarum* L.[27,28].

In the same family (Fabaceae), the study conducted on some Algerian populations of *Hedysarum coronarium* L., indicated that there is a link between the nitrogen (N) and the maximum height of plants during the first year of growth of this biennial plant[15].

As crude fiber, the ADF of legumes has a higher lignin than grasses and a lower cellulose content[29]. The greater

contribution of legume species to herbage production is indicated by Zarovali *et al.*[19]. In subhumid Mediterranean climate, the combination of subterranean clover with perennial grass can constitute a balanced pasture in quality[30].

CONCLUSION

The study of the behaviour and the chemical composition of Algerian populations of *Trifolium subterraneum* L., showed some variation between the different populations. Many relationships between the variables linked to the vegetative development and the chemical composition were highlighted. At this level, it seems that there is no link between the ecological factors (Altitude and rainfall) of the natural habitats and the variables linked to the vegetative development, the flowering, the formation of pods and the chemical composition of the different populations.

Investigations should be continued on a larger number of Algerian populations to know more about the behaviour and the chemical composition of subterraneum clover at different stages (different cutting dates) for a better understanding of the nature of variation signalized in this species (*Trifolium subterraneum* L.).

This study also would permit a better understanding of the source of variation established within the different species of the genus *Trifolium* L. and to compare and determine, which are the variables the most interesting in term of development and precocity and the most influenced by the ecological factors of the natural habitats within the different genera of the Fabaceae family (particularly the genera *Trifolium* L. and *Hedysarum*).

This study would contribute to the introduction of the best populations in a programme of selection for increasing the number of fodder legumes and developing the livestock, in the different agro-edapho-climatic areas, specially in the Mountains of the North East of Algeria, which is the most watered zone in the country.

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