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ANALYSIS OF THE MORPHOLOGICAL, PHENOLOGICAL AND BIOMETRICAL DIVERSITY IN SEVERAL ALGERIAN POPULATIONS OF *TRIFOLIUM SUBTERRANEUM* L. (*FABACEAE*)

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Abstract

As part of the evaluation and the valorization of plant genetic resources of fodder and pastoral interest in Algeria, twenty-six (26) natural populations of subterranean clover (*Trifolium subterraneum* L.), originating from different ecological habitats, were the subject of a morphological, phenological and biometrical study. In total, fifty (50) characteristics were considered (33 quantitative related to vegetative development, flowering, pods and 17 qualitative related to leaves, flowers and seeds). Two ecological factors (altitude and rainfall), concerning the natural habitats of the populations, were also considered. The analysis of variance highlighted the existence of significant intraspecific and inter-population variation for several traits. The Biometrical and phenological characteristics were more discriminating than qualitative ones (markers). Several significant correlations were observed between the markers and certain traits related to the reproduction and yield. Compared to the rainfall, the effect of the altitude was relatively more pronounced on the variation of certain quantitative and qualitative characteristics (seeds weight/fruiting heads weight ratio, flush intensity, crescent breadth and stem hairiness). The results of the PCA indicate a wide morpho-phenological and biometrical variation, particularly between the populations coming from the region of Guelma. The precocity, the vegetative development, the flower production and the fruiting heads weight permitted to classify the populations into three distinct groups. This study highlighted the importance of certain quantitative and qualitative characteristics in the infraspecific classification of the species *T. subterraneum* in Algeria and suggests that the populations collected in the Northeast of the country would correspond to the ssp. *subterraneum*. The diversified performances described in the Algerian populations of *T. subterraneum* require their preservation and valorization.

Key words: Clover, Diversity, Ecological factors, Infraspecific classification, Morphology, *Trifolium subterraneum* L.

Introduction

Subterranean clover (*Trifolium subterraneum* L., Sect. *Trichocephalum*), commonly known as burial clover or sowing clover, is a self-pollinating winter annual legume characterized by its hard seeds and its capacity to bury the reproductive structures in the soil ensuring thus, its own regeneration by self-seedling (Masson, 1997). Native to the Mediterranean Basin, West Asia and the Atlantic coast of Western Europe (Gladstones and Collins, 1983; Zohary & Heller 1984), subterranean clover is a major constituent of natural pastures in these regions (Zohary & Heller, 1984; Nichols *et al*., 2012, 2013), and has been found in areas ranging from 50 to 1550 mm of annual precipitation, and at altitudes from 0 to 2950 m (Ghamkhar *et al*., 2015). Subterranean clover has also been introduced to other countries with Mediterranean climates, including South Africa, Chile, Argentina, the West Coast and Gulf of Mexico, and parts of New Zealand and Uruguay (McGuire, 1985; Nichols *et al*., 2013; Abdi *et al*., 2020). Among annual clovers, subterranean clover makes the greatest contribution globally to livestock feed production and soi improvement (Mc Guire, 1985). In Australia, subterranean clover is highly valued for livestock, grains production, as a source of high-quality forage, and for its ability to fix atmospheric nitrogen (Nichols *et al*., 2013). Since its introduction and naturalization in 1900, it has become the most widely sown annual pasture legume particularly, in southern Australia, where it has been cultived over 29 million ha in southern Australia and 53 cultivars have been developed (Nichols *et al*., 2014). The adaptation of subterranean clover to production systems in this region and its success is attributed to its persistence and productivity (Nichols *et al*., 2012, 2013).

The species *T. subterraneum* constitutes a heterogeneous complex, divided into three subspecies: *subterraneum*, *brachycalycinum* and *yanninicum*, fairly identifiable by their morpho-physiology, karyotypes, isozymes, polymorphisms for molecular markers (Piluzza *et al*., 2005) and edaphic preferences (Masson, 1997). These three subspecies frequently occur in sympatry, but often have different boundaries (Piano *et al*., 1982).

In Algeria, this species was described by Quezel & Santa (1962), widespread in the Tell and mountain meadows. It is well adapted to non-saline and calcareous soils with various textures and variable pH (Issolah *et al*., 2015). Some populations of *T. subterraneum* have also been encountered on highly alkaline soils in North- East Algeria (Issolah *et al*., 2015).

Because of its importance in several countries around the world, *T. subterraneum* has been the subject of various studies (Nichols *et al*., 2009; Ghamkhar *et al*., 2016; Pazos-Navarro *et al*., 2017; Radecetti *et al*., 2018; Vasileva *et al*., 2020; Teixeira *et al*., 2020; Enkhbat *et al*., 2021a; Guo *et al*., 2022). Algerian populations of *T. subterraneum* have been used to enrich numerous international gene banks and to develop many cultivars (Genesis, 2021). Nevertheless, this natural resource remains little studied in Algeria (Zatout, 1985; Abdelguerfi *et al*., 2006; Issolah *et al*., 2015; Issolah *et al*., 2016).

The use and exploitation of forage species requires in-depth knowledge of populations for breeding. Inexpensive and easy to study, morphological markers are the first markers used in characterizing plant genetic resources (Nadeem *et al*., 2018). Their importance in studying genetic diversity between and within populations has been reported in some species of the genus *Trifolium* (Dias *et al*., 2008; Tucak *et al*., 2009; Bennani *et al*., 2010; Asci *et al*., 2011; Issolah *et al*., 2012; Medoukali *et al*., 2015).

The present study focuses on the evaluation and valorization of plant genetic resources of fodder and pastoral interest in Algeria. It aims to analyze the morpho-phenological and biometrical diversity within the natural populations of *T. subterraneum*, originating from different natural habitats in the Northeast of Algeria, in order to provide new data to clarify the taxonomic status of Algerian populations. This Work follows the different studies conducted on this legume in Algeria (Issolah *et al*., 2015; Issolah *et al*., 2016; Issolah, 2018; Bouziane *et al*., 2019).

Material and Methods

Plant material: Plant germplasm was collected by the National Institute of Agronomic Research of Algeria (INRAA), in 2010. Twenty-six (26) natural populations of subterranean clover (*Trifolium subterraneum* L.) coming from the different eco-geographical regions of Northeastern Algeria (Issolah *et al*., 2015), were considered (Table 1).

Table 1. Ecological characteristics of the natural sites of twenty-six (26) Algerian populations belonging to *Trifolium subterraneum* L.

N of populations	Origin	Altitude (m)	Rainfall (mm)
2/10	Guelma	530	600
3/10	Guelma	570	600
4/10	Guelma	660	600
6/10	Guelma	625	537
7/10	Guelma	230	500
10/10	Guelma	640	650
11/10	Guelma	820	650
12/10	Guelma	170	600
13/10	Guelma	200	558
16/10	Guelma	530	558
17/10	Guelma	530	558
18/10	Tarf	610	661
19/10	Tarf	665	661
20/10	Tarf	555	661
21/10	Souk Ahras	585	800
22/10	Souk Ahras	950	800
23/10	Souk Ahras	1040	700
24/10	Souk Ahras	810	700
25/10	Souk Ahras	800	900
26/10	Souk Ahras	1110	700
27/10	Souk Ahras	740	700
29/10	Guelma	430	550
33/10	Skikda	110	562
37/10	Constantine	380	700
38/10	Skikda	410	609
39/10	Skikda	560	845

Source: Issolah *et al*., (2015)

Experimental protocol and studied characteristics: The trial was conducted during the 2013-2014 growing season, at the experimental station of Baraki, Algiers (INRAA). It is a subhumid climate zone, with average annual temperatures ranging from 7.76°C (m) to 29.82°C (M) and an annual rainfall of 517.6 mm. The soil is characterized by a clay-silt texture, with an alkaline pH (7.8). The scarified seeds were manually sown on 9 December 2013, with thirty (30) seeds per line (30 individuals per population), in a randomized complete block design with three replications. The trial was conducted under rainfed conditions.

During this study, twenty-three (23) quantitative morpho-phenological characteristics (including three deduced) were considered: date of seedling emergence (DSE); winter development in height (WDH), winter development in width (WDW); spring development in height (SDH); spring development in width (SDW); number of branches (NB) (per most vigorous branch); leaflet length (LL); leaflet width (LW); petiole length (PL); peduncle length (PDL) (the last four traits are recorded for the node of the first flower); internode length (IL) (after the first flower); flowering and fruiting heads formation dates were expressed in number of days since emergence: flower bud (FB); first flower appearance (1F); full flowering (FF); end of flowering (EF); first fruiting head appearance (1FH); full formation of fruiting heads (FFH); start of fruiting heads maturity (SM); total drying of the plant (TD); number of flowers per plant (NFP). Three traits were deduced: winter growth speed in width (WGS); spring growth speed in width (SGS); duration of flowering (DF).

After the harvest, thirty (30) fruiting heads, were randomly selected for each population. Ten quantitative traits related to the fruiting heads, pods and seeds were measured: weight of 30 fruiting heads (WTH); fruiting head size: length (LFH) and width (WFH); number of pods per fruiting head (PFH); number of seeds per fruiting head (SFH); number of seeds per pod (SP); weight of seeds / weight of the fruiting heads ratio (RSF); thousand seed weight (TSW); seed size: length (LS), width (WS). For seed size, we considered the averages (length and width) of three seeds per fruiting heads (90 seeds per population), taken out randomly.

Furthermore, seventeen (17) qualitative morphological markers were noted on the same plants, during the winter and spring periods: leaf marks (crescent width, CW; band breadth, BB; arm breadth, AB; arm color, AC); leaflet indentation of the distal margin (LID); Intensity of leaflet anthocyanin pigmentation (fleck, FLE; flush, FLU); the degree of stipule anthocyanin pigmentation (SP); calyx tube pigmentation (CTP); degree of hairiness of the following parts: leaf upper surfaces (LUS), petioles (PH), peduncles (PDH), stems (RH); seed color (SC). Most of the traits were used from descriptors established for subterranean clover (Nichols *et al*., 1996; Ghamkhar *et al*., 2015). For the traits: leaf shape (LSH), seed shape (SSH) and burr burial ability (BBA), we adopted the following rating scale, respectively: 1 (triangular); 2 (triangular to round); 3 (round) (Anon., 2001) and 1 (round); 2 (round to elongate); 3 (flattened); 0 (no burial); 1 (presence of burial) (Nichols *et al*., 2013). Thus, seed shape and degree of burial can be used to separate the three subspecies of *T. subterraneum*: ssp. *subterraneum*, ssp. *brachycalycinum* and ssp. *yanninicum* (Katznelson & Morley 1965 *In* Nichols *et al*., 2013).

Statistical analyses

The quantitative data obtained were subjected to analysis of variance (ANOVA) and Tukey's test ($p\leq0.05$). The correlations between the different characteristics and the ecological factors of the natural habitat of the populations (Altitude, ALT and rainfall, PLU) were performed. The structure of phenotypic variability was studied using a principal component analysis (PCA) to determine the grouping of populations. These statistical treatments were made thanks to MINITAB 2016 and XLSTAT 2016 softwares. For the different qualitative markers, the diversity index (H') of Shannon-Weaver (Hennink & Zeven, 1991) was determined: $H' = H/Hmax$, where $H = -\sum_{i=1}^{n} Pi Ln Pi$ and $Hmax = ln(n)$.

The diversity index was considered low ($0.10 \leq H' \leq 0.40$), intermediate ($0.40 \leq H' \leq 0.60$), or high ($H' \geq 0.60$) (Eticha *et al*., 2005).

Results and Discussion

Variance analysis: The variance analysis revealed a large variation within the Algerian populations of *T. subterraneum*. In fact, very highly significant differences ($p\leq0.001$) were noted for most of the quantitative morpho-phenological and biometrical characteristics, except for the internode length (LEN) which showed a non-significant effect (Table 2). The obtained results indicated that all the populations showed clearly, a vegetative development better in spring than that observed in winter (Table 2). Some populations (4/10, 37/10 and 19/10) were distinguished by good spring vegetative development, particularly in width (SDW), with a rapid growth speed (SGS). The first two populations (4/10 and 37/10) also expressed the highest values for the number of branches (NB) (Table 2). This performance observed in Algerian populations and linked to the vegetative development, particularly the soil covering in spring, could be selected to improve the grazing aspect and consequently, the livestock.

Similar results have been advanced in different studies concerning the behaviour in natural Algerian populations (Issolah *et al*., 2016), French populations (Masson *et al*., 1996), a core collection and Australian varieties (Abdi *et al*., 2020) of *T. subterraneum*. The same finding has been reported through several studies conducted on natural populations of the genus *Trifolium* in Algeria (Issolah *et al*., 2006) and Morocco (Bennani *et al*., 2010).

Thus, significant variation was found for flowering traits and pod formation (Table 2). Some populations (12/10 and 37/10) were the earliest for flowering traits (flower bud, FB; first flower appearance, 1F). The first population (12/10) was also early for the formation of fruiting heads (first fruiting head, 1FH; fruiting heads maturity, SM). However, the population 37/10 presented an early full fruiting heads formation (FFH), an early fruiting heads maturity (SM) and gave a high number of flowers per plant (NFP) (Table 2). The population 38/10 was the latest for the flowering and the fruiting heads formation and presented the shortest flowering duration (DF) (Table 2). Flowering time is generally recognized as a major trait affecting genotype and population adaptation (Wallace *et al*., 1993). In subterranean clover, this trait has long been found to be strongly related to the success of genotypes in given environments (Rossiter, 1966). Concerning the appearance of the first flower, our results seem to be close to those reported by Issolah *et al*., (2016) about ten Algerian populations of *T. subterraneum*. Thus, several studies conducted on collections of *T. subterraneum*, in Italy (Piano, 1984; Piano *et al*., 1993; Piano & Pecetti, 1996; Pecetti & Piano, 2002), France (Masson *et al*., 1996), Australia (Ghamkhar *et al*., 2015; Abdi *et al*., 2020) and Germany (Baresel *et al*., 2018), have shown wide diversity for flowering time.

The characteristics related to the fruiting heads, pods and seeds were highly variable and more discriminating compared to other ones (Table 2). Different studies conducted on genetic diversity within Portuguese populations of *T. subterraneum* (Goulão *et al*., 1999) and between different *Trifolium* species (Issolah, 2006; Medoukali *et al*., 2015) have reported the importance of pod and seed characteristics in intra- and inter-specific variation.

The results obtained through the present study indicated that Algerian populations of *Trifolium subterraneum* have relatively smaller fruiting heads compared to those of some *species* corresponding to the same genus. A study conducted on twelve species of *Trifolium*, showed that the species *T. spumosum*, *T. Repens*, *T. Bocconei* Savi. and *T. fragiferum*, have large fruiting heads (Issolah & Abdelguerfi, 1995). Concerning the length of the fruiting heads (LFH) recorded in the Algerian populations of *T. subterraneum*, our results indicated that it was slightly close to the length reported by Zohary & Heller (1984) and relatively lower than that reported by Porqueddu *et al*., (2003).

According to Collins *et al*., (1976) and Pecetti & Piano (1994), the small size of subterranean clover fruiting heads, promotes their burial in the soil, allows normal seed development, and also facilitates its self-seeding (Bullita *et al*., 1994). Thus, the results of this study reveal that population 17/10 had the highest average number of pods (PFH) and seeds per fruiting head (SFH) (Table 2). The population 26/10 was distinguished by its large seeds (LS, WS), higher seeds to fruiting heads weight ratio (RSF) and consequently, higher thousand seed weight (TSW) (Tables 2 and 3). The average number of seeds per fruiting head recorded in Algerian populations is slightly higher than that reported by Goulão *et al*., (1999) for Portuguese populations and varieties of *T. subterraneum*, as well as that reported by Mackay (1981) for Australian cultivars and Norman *et al*., (2005) for Mediterranean and Australian accessions. In the same context, Zohary & Heller (1984) showed in varieties (subspecies) of *T. subterraneum*, the presence of flower heads containing 4-6 flowers and others with 2, 3, and 4 flowers. Quezel & Santa (1962) also reported 2-7 flowers.

In addition to the inter-population variation noted for the average number of pods and seeds per fruiting head, an intra-population variation was clearly noticed for these characteristics in some individuals within the same population, particularly, the populations 26/10, 25/10 and

19/10. In fact, within these populations, the individuals presented respectively, a maximum of eight (08), nine (09), and up to ten (10) pods and seeds per fruiting head. The variation observed in these populations requires special attention to be selected and exploited in the development of seed production within *T. subterraneum*. For the number of seeds per pod, the result of this study is agree with those of Zohary & Heller (1984), who reported the presence of one seed per pod in subterranean clover. In our case, the number of two (2) seeds per pod was observed only in some individuals of the Algerian populations (18/10, 20/10 and 26/10).

Concerning the seed size (LS and WS), the obtained results indicated that it is relatively larger in Algerian populations compared to that advanced in American specimens of *T. subterraneum* (Zoric *et al*., 2010). In their study on the morphometry of *Trifolium* seeds, Zoric *et al*., (2010) found that the size and weight of *T. subterraneum* seeds were larger than those of other *Trifolium* species. These exceptional seed characteristics could be explained by the specific conditions of fruiting and seed development under the soil (Zoric *et al*., 2010). Large seeds provide a competitive advantage during seedling establishment (Carleton & Cooper, 1972; Venable, 1992). However, Cocks *et al*., (1982) found that successful genotypes of ssp. *subterraneum* tended to have small seeds. According to Russi *et al*., (1992), clovers and annual medics with small seeds have lower degradation of seed coat impermeability induced by temperature fluctuations compared to species with large seeds.

The thousand seed weight gives a priori idea about the seed size (Lesins & Lesins, 1979). Our results indicated that the thousand seed weight (TSW) of populations is lightly higher than that reported by Issolah *et al*., (2016) in Algerian populations, Varis *et al*., (1990) in some Australian lines and Zoric *et al*., (2010) in American specimens of *T. subterraneum*.

Table 2. Results of the variance analysis of morpho-phenological and biometrical characteristics within twenty-six (26) Algerian populations of *Trifolium subterraneum* L.

Characteristics	Min	Max	Mean	Fobs	Probability
DSE (d)	20	34.13	27.11^{abcd}	3.06	0.000***
WDH (cm)	1.59	2.97	2.23^{abc}	2.49	0.000***
WDW (cm)	6.43	13.97	9.04^{abcd}	3.30	0.000***
SDH (cm)	3.24	5.14	3.99^{ab}	1.7	0.02*
SDW (cm)	54.91	108.67	78.79^{abcde}	3.7	0.000***
LL (cm)	1.07	1.35	1.22^{a}	1.68	0.023*
LW (cm)	1.10	1.43	1.28^{ab}	2.11	0.002**
PL (cm)	2.74	4.39	3.63^{ab}	2.20	0.001***
PDL (cm)	2.71	4.53	3.48^{ab}	2.39	0.000***
IL (cm)	3.80	5.51	4.62	0.82	0.697^{NS}
NB	3.53	6.73	5.43^{ab}	1.96	0.005**
SGS (cm/d)	0.08	0.22	0.13^{abcd}	3.07	0.000***
WGS (cm/d)	0.78	1.60	1.16^{abcd}	3.38	0.000***
FB (d)	96.83	111.47	102.58^{ab}	2.86	0.000***
1F (d)	101	115.4	106.54^{abc}	2.98	0.000***
FF (d)	114.53	128	119.79^{ab}	2.05	0.002**
EF (d)	142.87	162.6	150^{abc}	4.19	0.000***
1FH (d)	114.07	128.93	120.14^{abcd}	3.00	0.000***
FFH (d)	143.13	161.67	149.90^{abc}	3.92	0.000***
SM (d)	136.6	152	144.99^{abcd}	2.84	0.000***
TD (d)	159.87	173.73	167.58^{ab}	2.58	0.000***
NFP	61.18	143.8	92.88^{ab}	2.49	0.000***
FD (d)	37.2	56.6	43.49^{abcdef}	6.27	0.000***
LFH (cm)	0.71	0.92	$0.78^{abcdefghi}$	12.81	0.000***
WFH (cm)	0.74	0.88	0.82^{abcdef}	5.55	0.000***
PFH	3.83	5.10	4.41^{abcde}	3.56	0.000***
SFH	3.77	5.03	4.37^{abcde}	3.53	0.000***
SP	1	2	-	-	-
LS (mm)	2.68	3.35	$2.96^{abcdefghij}$	9.41	0.000***
WS (mm)	1.69	2.16	$1.87^{abcdefghij}$	9.55	0.000***

Min, Max: Minimum and maximum mean of a population, Mean: Average of the species, DSE: Date of seedling emergence, WDH: Winter development in height, WDW: Winter development in width, SDH: Spring development in height, SDW: Spring development in width, LL: Leaflet length, LW: Leaflet width, PL: Petiole length, PDL: Peduncle length, NB: Number of branches (per most vigorous branch), IL: Internode length, WGS: Winter growth rate in width, SGS: The spring growth rate in width, FB: Flower bud, 1F: First flower appearance, FF: Full flowering, EF: End of flowering, 1FH: First fruiting head appearance, FFH: Full formation of fruiting heads, SM: Start of fruiting heads maturity, TD: Total desiccation, NFP: Number of flowers per plant, DF: Duration of flowering, LFH: Length of the fruiting head, WFH : Width of the fruiting head, PFH: Number of pods per fruiting head, SFH: Number of seeds per fruiting head, SP: number of seeds per pod, LS: Length of seed, WS: Width of seed. Signification: p*≤0.05; p**≤0.01; p***≤0.001; NS: No significant

Table 3. Variation of some statistical parameters within twenty-six (26) Algerian populations of *Trifolium subterraneum* L.

Parameters	Min	Max	Mean	S.D	C.V (%)
WTH (g)	2.77	5.42	4.08	0.62	15.10
RSF	0.15	0.36	0.24	0.04	15.91
TSW (g)	4.42	9.56	7.01	1.25	17.77

Min, Max: Minimum and maximum mean of a population, Mean: Mean of the species, WTH: Weight of 30 fruiting heads, RSF: Weight of seeds to the weight of the fruiting heads ratio, TSW: Thousand seed weight, S.D: Standard deviation, C.V: Coefficient of variation

Furthermore, the results concerning qualitative morphological markers reveal that a remarkable phenotypic diversity characterizes the Algerian populations of *T. subterraneum* (Table 4). According to Shannon-Weaver diversity index (H'), this variation seems to be enough accentuated for the vegetative characteristics of leaves (shape, leaf marks and pigmentation), petioles, stems, floral peduncles and stipules compared to those of flowers and seeds (Table 4). The highest values of this index were observed for the shape of the leaves and anthocyanin coloration of the stipules, while the lowest values were established for the pigmentation of the calyx tube as well as the color of the seeds (Table 4).

In terms of vegetative growth, the populations of *T. subterraneum* are mostly characterized by triangular to round, very weakly toothed at their distal margin, weakly hairy on their upper surfaces. Each of the three leaflets (trifoliates) has a particular leaf mark (Tables 4 and 5).

Only three classes of leaf marks were found in the Algerian populations of *T. subterraneum*: "crescent + arms", "arms only", "no marks". None of them showed leaf marks in the form of crescents alone or bands. In fact, the leaf mark "crescent + arms" was the most frequently observed during this study on the leaves of Algerian populations (Tables 4 and 5). This is consistent with the results reported by Pecetti & Piano (2002) on Italian populations and Abdi *et al*. (2020) on a core collection and cultivars of *T. subterraneum*. It has been shown that the leaf mark trait in *T. subterraneum* is highly heritable and the most distinctive and variable morphological characteristic (Nichols *et al*., 1996; Nichols *et al*., 2013). In the same species (*T. subterraneum*), each leaf mark is controlled by a single allele at a single locus that has over thirty different alleles (Tan and Collins, 1987; Ghamkhar *et al*., 2012).

For the pigmentation of the calyx tube, the results show that it was similarly distributed on the upper quarter (¼) of the calyx in the most studied Algerian populations (Tables 4 and 5), which seems to be different from the results obtained by Pecetti & Piano (2002) on Italian populations, most of whom showed no pigmentation. According to Nichols *et al*., (2013), the pigmentation of the calyx tube is very discriminating between the two subspecies of *T. subterraneum* (ssp. *subterraneum* and ssp. *brachycalycinum*), in which the ssp. *brachycalycinum* never presents it.

On the other hand, the shape of the seeds of the Algerian populations ranged from round to elongated (Tables 4 and 5). No population presented the flattened shape. The latter shape is specific to ssp. *brachycalycinum* (Nichols *et al*., 2013).

Seed traits are widely used in seed certification and cultivar identification (Nichols *et al*., 1996) and also for distinction between the three subspecies of *T. subterraneum* (ssp. *subterraneum*, ssp. *brachycalycinum* and ssp. *yanninicum*) (Nichols *et al*., 2013).

We also noted that all the studied populations had short peduncles (less than 5 cm), a strong geotropism and a high capacity to bury their fruiting heads in the soil.

Pilluza *et al*. (2005) reported that the morphological differences between the three subspecies (ssp. *subterraneum*, ssp. *brachycalycinum* and ssp. *yanninicum*) of *T. subterraneum* are very remarkable and discernable.

In some regions of Algeria, *Trifolium subterraneum* L. is represented by three varieties (Subsp. *subterraneum* Var. *subterraneum;* Var. *brachycladum;* Var. *flagelliforme*) out of the eight varieties (Subsp. *subterraneum* Var. *subterraneum;* Var. *brachycladum;* Var. *majurculum;* Subsp. *brachycalycinum* Var. *brachycalycinum;* Var *graecum;* Var. *flagelliforme;* Var. *oxaloides;* Var. *yanninicum*) described in this species (Zohary & Heller, 1984).

Dobignard & Chatelain (2012) and the flora of the Magreb (eflore) (Chatelain *et al.*, 2018) reported that the species *T. subterraneum* is represented in Algeria by two subspecies: ssp. *subterraneum* and ssp. *oxaloides*.

On the other hand, several accessions corresponding to *T. subterraneum* ssp. *brachycalycinum* Katzn. & Morley have been identified from different regions of Algeria (Relizane, Blida, Médéa, Skikda, Oum El Bouaghi) (Genesis, 2021).

Recently, *T. subterraneum* ssp. *subterraneum* is described by the following heterotypic synonym: *Trifolium subterraneum* var. *brachycladum* Gibelli & Belli (African Plant Database, 2021). *T. subterraneum* ssp. oxaloides is also described by the following heterotypic synonyms: *T. subterraneum* ssp. b*rachycalycinum* Katzn. & Morley (1965) and *Trifolium subterraneum* var. *flagelliforme* Guss. (1854) (African Plant Database, 2021).

The characteristics of *T. subterraneum* ssp. *subterraneum* were reported by several authors (Nichols *et al*., 1996, 2013; Ghamkhar *et al*., 2015).

On the basis of the results obtained through the present study and linked to several qualitative and quantitative morphological characteristics, particularly those concerning the presence of anthocyanic pigmentation on the calyx tube, the shape of the seeds, the good performance of burial of the fruiting heads in the soil, the rather short size of the floral peduncles (not exceeding 5 cm) and the internodes, we can suggest that the populations of *T. subterraneum*, met in different regions of the Northeastern Algeria (Guelma, Tarf, Souk Ahras, Skikda, Constantine), would correspond to the ssp. *subterraneum*.

Table 4. Phenotypic classes and diversity indices of qualitative morphological markers within twenty-six (26) Algerian populations of the species *Trifolium subterraneum* L.

Morphological markers	Phenotypic classes	Number of classes reached /Rating scales	Classes proportion (%)	Diversity index of shannon-weaver (H')
Vegetative characteristics				
Leaf Shape	Triangular Triangular - rounded Rounded	3/3	23.08 53.85 23.08	0. 919
Indentation of the distal Margin	Absent or very weak Medium Strong	3/3	65.38 26.92 7.69	0.754
Hairiness of upper Surfaces	Absent or very weak Weak Medium	3/5	26.92 53.85 19.23	0.623
Hairiness of petiole	Weak Medium Strong	3/5	53.85 38.46 7.69	0.558
Leaf marks				
Width of crescent pale green	Absent C1 C2 C3	4/5	34.62 7.69 42.31 15.38	0.745
Breadth of arms	Absent A1 A2	¾	19.23 61.54 19.23	0.673
Color of arms	Absent Pale green White	3/3	19.23 53.85 26.92	0.750
Anthocyanin pigmentation of the leaflet				
Flush	Absent or very weak Weak Strong	¾	11.53 11.53 50	0.609
Fleck	Absent or very weak Weak Medium	¾	59.69 38.46 7.69	0.616
Anthocyanin pigmentation of stipules	Weak Medium Strong	¾	34.62 30.76 34.62	0.791
Hairiness of stems	Weak Medium Strong	3/5	30.77 50 19.29	0.613
Flowers				
Hairiness of peduncle	Weak Medium Strong	3/5	26.92 42.31 30.77	0.669
Anthocyan in pigmentation of tube calyx	¼ upper of calyx tube ½ upper of calyx tube	2/5	73.08 15.38	0.336
Seeds				
Shape	Rounded Rounded - Elonged	2/3	46.15 57.67	0.613
Color	Brown Black	2/3	92.31 7.69	0.247

C1: Small dot; C2: Extending approximately from center to halfway towards leaf margins; C3: Extending approximately from center to two-thirds distance towards leaf margins; A1: Narrow arms; A2: Intermediate arms; A3: Thick arms; ¼ upper of calyx tube: On the upper quarter of calyx tube; ½ upper of calyx tube: On the upper half of calyx tube

Table 5. Qualitative morphological markers found within twenty-six (26) Algerian populations of the species *Trifolium subterraneum* L.

Markers / Scales	Leaf			Leaf marks			Anthocyanin pigmentation (Leaf; Stipules; Flowers)				Hairiness			Seeds		Fruiting heads
Populations	Shape of leaf (1-3)	Indentation (Leaflet distal margin) (0-4)	Hairiness of upper surfaces) (0-8)	Crescent width (Absent-C4)	Arms Breadth (Absent -A_3)	Arms Colour	Flush (0-6)	Fleck (0-6)	Stipules (0-6)	Calyx tube (0-8)	Stem (0-8)	Petiole (0-8)	Peduncle (0-8)	Shape (1-3)	Colour (1-3)	Burialability (0-1)
2/10	2	0	2	C2	A1	Pale green	6	0	2	2	2	2	2	2	Brown	1
3/10	1	2	2	C2	A1	White	6	0	4	4	2	2	2	2	Brown	1
4/10	2	0	2	C2	A1	White	6	2	6	2	4	4	2	2	Brown	1
6/10	2	2	2	C2	A2	Pale green	2	4	4	2	4	2	4	2	Brown	1
7/10	3	4	2	Absent	Absent	-	6	2	6	2	6	2	4	1	Brown	1
10/10	3	0	2		A1	Pale green	6	2	4	2	4	2	2	1	Brown	1
11/10	3	0	2	Absent	Absent	-	6	2	6	2	4	4	4	1	Brown	1
12/10	2	0	2	C2	A2	Pale green	6	2	6	2	4	4	4	2	Brown	1
13/10	3	0	2	C2	A2	Pale green	6	2	6	2	6	4	4	2	Brown	1
16/10	2	0	2	C1	A1	White	4	0	2	2	4	2	6	2	Brown	1
17/10	2	0	2		A1	Pale green	4	2	4	2	4	6	6	2	Brown	1
18/10	2	4	2	Absent	Absent	-	4	0	6	2	6	2	2	1	Brown	1
19/10	2	0	2	C2	A2	Pale green	4	4	6	4	2	2	6	2	Brown	1
20/10	2	0	2	Absent	Absent	-	4	2	2	4	4	2	2	2	Brown	1
21/10	2	0	4	C2	A1	Pale green	4	2	6	2	4	4	4	2	Marron	1
22/10	2	0	2	C3	A1	Pale green	2	0	4	2	2	2	4	1	Black	1
23/10	2	0	2	C2	A1	Pale green	2	2	2	2	2	4	6	2	Brown	1
24/10	1	0	2	C1	A1	White	6	0	2	2	2	2	4	2	Brown	1
25/10	3	0	4	C3	A1	Pale green	2	0	6	2	4	4	4	2	Brown	1
26/10	2	0	2	C3	A1	White	2	0	4	2	4	2	4	2	Brown	1
27/10	1	2	4	C3	A1	White	2	0	2	2	4	2	4	1	Brown	1
29/10	3	0	4	Absent	Absent	-	6	0	4	2	6	4	6	1	Brown	1
33/10	1	0	4		A1	Pale green	4	0	2	2	6	4	6	1	Black	1
37/10	1	0	2		A1	Pale green	6	0	4	4	4	6	6	1	Brown	1
38/10	1	2	2	C2	A1	White	6	0	2	2	2	4	6	1	Brown	1
39/10	1	2	2	C2	A2	White	6	0	2	2	2	2	2	1	Brown	1

C1: Small dot; C2: Extending approximately from center to halfway Towards leaf margins; C3: Extending approximately from center to two-thirds distance towards leaf margins; A1: Narrow arms; A2: Intermediate arms; A3: Thick arm

Relationships between the morphological characteristics and the ecological factors of the natural habitats: Several significant correlations were noted between all the traits, on the one hand, and the factors of the environment of origin, on the other hand (Tables 6 and 7).

The characteristics related to vegetative development are strongly correlated with each other.

Thus, populations with good winter development in width (WDW) also showed good spring development in height and width (SDH; SDW). These populations also presented the most accelerated growth speed in winter and spring (WGS; SGS), high numbers of branches (NB) and flowers per plant (NFP).

The populations characterized by good winter development in height (WDH) are late to flowering and fruiting heads formation. They produce more pods (PFH) and seeds (SFH) per fruiting head.

Early flowering populations are also early in the production of fruiting heads. These are the most late-emerging populations (DSE).

The populations with high fruiting heads weight (WTH) have wider fruiting heads (WFH), larger seeds (LS; WS) and high thousand-seed weight (TSW). They are more performant in terms of spring development in height and width and give a high number of flowers per plant.

The populations with high intensity of flush (FLU), are characterized by good spring development in width (SDW), an accelerated growth speed in spring (SGS), relatively long petioles (PL), a late flower bud release (FB), and a heavier fruiting head (WTH). These populations come from low altitude areas.

The populations with highly anthocyanin-stained stipules (SP), are characterized by a good spring growth speed in width (SGS) and a long flowering duration (DF). The populations with a more or less round leaf shape (LSH) are the latest at the end of flowering (EF), full fruiting heads (FFH) and give more pods (PFH) and seeds (SFH) per fruiting head.

We noticed that the several characteristics related to vegetative development were associated with different studied qualitative markers, compared to reproductive and production characteristics. Nevertheless, the correlations found for some characteristics related to reproduction and production, notably, the end of flowering, the duration of flowering, the full flowering and the weight of the thirty fruiting heads were highly significant.

Concerning the relationships with the factors of the environment of origin, the correlation matrix highlighted significant relationships between certain morphological characteristics (quantitative and qualitative) and the ecological factors of the natural habitats (Altitude and Rainfall) of the populations (Tables 6 and 7). In fact, altitude affects the ratio of seeds weight/ fruiting heads weight (RSF, quantitative) and leaflet flushing (FLU, qualitative). Thus, populations originating from higher altitudes areas have a high seeds weight/fruiting heads weight ratio and a low flushing intensity on their leaves (Tables 6 and 7).

On the other hand, rainfall influences the winter development in height (WDH). The populations coming from the rainiest regions are the least vigorous in terms of height development at the beginning of the cycle. Lilley *et al*., (2001) reported that seed and biomass production in *T. subterraneum* are much more sensitive to environmental factors, while morphological characters such as plant height and leaf size are not very sensitive.

Only two qualitative characteristics (width of the leaf crescent, CW; hairiness of the stems, HS) are influenced by both factors of the environment of origin (altitude and rainfall). Populations coming from the most humid regions and high altitudes areas, showed wider leaf crescents and less hairy stems.

Evaluation of the relationships between variation in key adaptive traits of natural populations and the ecological characteristics of their sites of origin have provided selection models for the development of new cultivars in given regions (Piano & Pecetti, 1996).

In a study of ten Algerian natural populations of *T. subterraneum*, Issolah *et al*. (2016) reported that some ecological factors (Altitude and rainfall) did not influence any of the vegetative, reproductive and production traits, moreover, altitude has been shown to influence some physico-chemical characteristics of soils related to natural habitats of several populations of *T. subterraneum* (Issolah *et al*., 2015).

Within a core collection and cultivars of *T. subterraneum*, Abdi *et al*., (2020) confirmed that some specific characteristics of the plant are associated with environmental factors at their sites of origin and are therefore adaptive.

Flowering time is highly sensitive to latitude, temperature, winter and annual precipitation (Piano, 1984; Piano *et al*., 1993; Pecetti & Piano, 2002; Abdi *et al*., 2020; Enkhbat *et al*., 2021a). Ghamkhar *et al*. (2015) on their study of world collection of *T. subterraneum*, excludes the presence of correlation between flowering time and annual rainfall, however, several studies have reported the effects of certain ecological factors of origin environment on variation of morphological traits in *T. subterraneum*. Baresel *et al*. (2018) found that plants from warmer climates produce more biomass. More vigorous winter growth in subterranean clover appears to be related to lower latitude regions, and peduncle length is associated with increasing soil pH (Abdi *et al*., 2020).

In *T. subterraneum* ssp. *yanninicum*, Enkhbat *et al*. (2021a) reported that high altitudes and summer precipitation favoured late-flowering genotypes with large leaves, long petioles and long internodes at first flowering. Thus, in the same subspecies (ssp. *yanninicum*), annual mean temperature, winter and summer temperatures influence flowering time, petiole and internode lengths (Enkhbat *et al*., 2021a).

On the other hand, relationships have been reported in subterranean clover between arm breadth of leaf marks, anthocyanin pigmentation, pilosity traits, rainfall, latitude, and soil pH (Abdi *et al*., 2020). Arm breadth of leaf marks was associated with both altitude, longitude, mean annual temperature, and winter and summer temperatures in *T. subterraneum* ssp. *yanninicum* (Enkhbat *et al*., 2021a). No study found an association between crescent width and eco-geographic parameters of natural habitats (Abdi *et al*., 2020; Enkhbat *et al*., 2021a).

Table 6. Pearson's correlation between quantitative characteristics and the ecological factors of the natural habitats within twenty-six (26) Algerian populations of *Trifolium subterraneum* L.

Characteristics	WDH	WDW	SDW	SGS	FF	DF	1FH	FFH	TD	NFP	LL	L PDL	PFH	LS	WS	WTH	RSF
DSE	-0.40*	-0.46*	-0.28	-0.23	-0.82***	0.01	-0.79**	-0.49*	-0.80***	-0.36	-0.06	-0.04	0.00	0.25	0.08	-0.11	0.21
SDH	-0.01	0.52**	0.67***	0.66***	-0.03	0.14	0.01	-0.05	-0.05	0.64***	0.00	-0.22	-0.04	0.48*	0.29	0.49*	-0.12
SDW	0.23	0.66***	-	0.99***	0.19	0.16	0.17	0.21	0.25	0.59***	0.38	0.14	0.22	0.13	0.07	0.63***	-0.41
WGS	-0.05	0.89***	0.56**	0.44*	-0.02	0.16	-0.11	-0.02	0.15	0.44*	0.28	0.10	-0.04	0.14	0.02	0.13	0.01
SGS	0.24	0.55**	0.99***	-	0.18	0.16	0.18	0.23	0.22	0.56**	0.37	0.13	0.25	0.12	0.07	0.66***	-0.44*
1F	0.57***	-0.02	0.07	0.09	0.93***	-0.35	0.88***	0.38	0.67***	0.00	0.07	0.14	0.20	-0.34	-0.03	0.05	-0.15
FF	0.53**	0.20	0.19	0.18	-	-0.26	0.91***	0.39*	0.75***	0.07	0.08	0.07	0.14	-0.32	-0.06	0.00	-0.10
EF	0.16	0.10	0.22	0.23	0.44*	0.71***	0.52**	0.95***	0.75***	0.13	0.07	-0.15	0.25	-0.14	0.09	0.12	-0.03
DF	-0.28	0.12	0.16	0.16	-0.26	-	-0.14	0.68***	0.26	0.13	0.02	-0.26	0.10	0.10	0.11	0.08	0.08
1FH	0.39	0.12	0.17	0.18	0.91***	-0.14	-	0.44*	0.77***	0.13	-0.07	-0.07	0.16	-0.14	0.14	0.10	-0.04
FFH	0.12	0.08	0.21	0.23	0.39*	0.68***	0.44*	-	0.66***	0.16	0.11	-0.09	0.21	-0.18	0.09	0.19	0.10
SM	0.41*	-0.03	0.10	0.11	0.82***	-0.23	0.85***	0.40*	0.77***	-0.10	0.11	0.16	0.24	-0.30	0.07	0.05	-0.01
TD	0.40*	0.29	0.25	0.22	0.75***	0.26	0.77***	0.66***	-	0.04	0.15	0.09	0.23	-0.27	-0.02	0.02	-0.06
NFP	-0.05	0.59***	0.59***	0.56**	0.07	0.13	0.13	0.16	0.04	-	0.01	-0.02	0.05	0.22	0.06	0.55**	-0.38
NB	-0.13	0.48*	0.36	0.30	0.13	0.15	0.11	0.14	0.30	0.33	0.13	-0.02	-0.13	0.03	0.10	0.16	0.02
LW	0.28	0.03	0.14	0.15	0.02	-0.24	-0.12	-0.10	-0.10	-0.07	0.71***	0.62***	0.34	-0.38	-0.42*	0.05	-0.25
IL	0.10	0.20	0.30	0.29	0.13	-0.11	-0.01	0.09	-0.03	0.43*	0.34	0.31	0.22	-0.19	-0.07	0.35	0.35
PL	0.15	0.14	0.15	0.15	0.03	-0.22	-0.02	-0.09	-0.32	0.18	0.26	0.47*	0.13	-0.25	-0.16	0.11	-0.24
LDL	0.45*	0.06	0.14	0.13	0.07	-0.26	-0.07	-0.09	0.09	-0.02	0.66***	-	0.40*	-0.53**	-0.43*	-0.01	0.29
LFH	-0.36	0.06	-0.14	-0.16	-0.20	0.41*	-0.16	0.26	0.01	-0.01	-0.21	-0.19	-0.30	0.13	0.12	-0.08	0.05
WFH	0.15	0.16	0.27	0.26	0.14	-0.14	0.14	0.08	0.10	0.34	-0.08	0.14	0.24	0.25	0.28	0.50**	-0.03
PFH	0.54**	-0.10	0.22	0.25	0.14	0.10	0.16	0.21	0.23	0.05	0.36	0.40*	-	-0.15	-0.04	0.06	0.12
SFH	0.52**	-0.16	0.13	0.16	0.15	0.09	0.14	0.22	0.19	0.02	0.34	0.38	0.97***	-0.17	-0.05	0.00	0.20
LS	-0.21	0.11	0.13	0.12	-0.32	0.10	-0.14	-0.18	-0.27	0.22	-0.32	-0.53**	-0.15	-	0.81***	0.49*	0.35
WTH	0.09	0.24	0.63***	0.66***	0.00	0.08	0.10	0.19	0.02	0.55**	0.17	-0.01	0.06	0.49*	0.49*	-	-0.44*
TSW	-0.05	0.26	0.31	0.29	-0.02	-0.14	0.07	-0.11	-0.10	0.25	-0.02	-0.21	-0.07	0.66***	0.77***	0.57***	0.31
ALT	-0.04	0.01	-0.22	-0.26	-0.25	0.12	-0.24	-0.08	-0.07	-0.10	-0.06	0.02	0.05	0.14	0.06	-0.38	0.48*
RNF	-0.43*	0.09	-0.24	-0.29	-0.18	0.27	-0.13	0.01	0,05	-0.19	-0.08	-0.03	-0.31	-0.05	-0.06	-0.37	0.35

DSE: Date of seedling emergence, WDH: Winter development in height, WDW: Winter development in width, SDH: Spring development in height, SDW: Spring development in width, LL: Leaflet length, LW: Leaflet width, PL: Petiole length, PDL: Peduncle length, NB: Number of branches (per most vigorous branch), IL: Internode length, WGS: Winter growth speed in width, SGS: Spring growth speed in width, 1F: First flower appearance, FF: Full flowering, EF: End of flowering, 1FH: First fruiting head appearance, FFH: Full formation of fruiting heads, SM: Start of fruiting heads maturity, TD: Total drying of the plant, NFP: Number of flowers per plant, DF, Duration of flowering, LFH: Length of the fruiting head, WFH: Width of the fruiting head, PFH: Number of pods per fruiting head, SFH: Number of seeds per fruiting head, SP: Number of seeds per pod, LS: Length of the seed, WS, The width of seed, WTH: Weight of 30 fruiting heads, RSF: Weight of seeds to the weight of the fruiting heads ratio, TSW: Thousand seed weight, ALT: Altitude, RNF: Rainfull,Probability (signification): $p^{*} \leq 0.05$, $p^{**} \leq 0.01$, $p^{***} \leq 0.001$

Table 7. Relationship between qualitative morphological markers, quantitative characteristics, and ecological factors of the natural habitats within twenty-six (26) Algerian populations of *Trifolium subterraneum* L.

Characteristics	LSH	CW	AB	AC	FLU	SP	CTP	PH	SH	PDH	SSH
DSE	-0.03	0.17	0.15	-0.06	-0.41*	0.23	0.03	0.03	0.04	0.00	0.23
WDH	0.13	-0.39	-0.41*	-0.05	0.30	-0.16	-0.03	-0.03	0.02	0.17	-0.27
SDH	-0.08	-0.06	0.09	0.27	0.40*	0.29	0.34	0.34	-0.23	0.03	0.12
SDW	0.07	-0.16	0.17	0.22	0.48**	0.44*	0.25	0.25	0.07	0.00	0.00
WGS	-0.19	0.22	0.26	0.41*	-0.01	0.01	0.44*	0.44*	-0.33	0.14	0.17
SGS	0.12	-0.21	0.15	0.17	0.50**	0.50**	0.21	0.21	0.13	-0.01	-0.01
FB	0.14	-0.16	-0.19	0.01	0.49**	-0.19	-0.33	-0.33	0.05	0.05	-0.28
1F	0.16	-0.17	-0.20	-0.01	0.43*	-0.19	-0.33	-0.37	0.08	0.08	-0.29
FF	0.15	-0.14	-0.15	0.02	0.40*	-0.12	-0.28	-0.28	0.06	0.21	-0.35
EF	0.58***	-0.09	-0.12	-0.26	0.12	0.41*	-0.09	-0.09	0.20	-0.01	0.07
DF	0.44*	0.04	0.04	-0.25	-0.21	0.57***	0.19	0.19	0.13	-0.08	0.29
1FH	0.26	-0.11	-0.13	-0.03	0.44*	0.02	-0.29	-0.29	0.10	-0.01	-0.16
FFH	0.62***	-0.21	-0.21	-0.39	0.14	0.46*	-0.14	-0.14	0.32	-0.04	-0.03
NFP	0.04	-0.28	-0.13	0.03	0.45*	0.10	0.30	0.30	-0.02	-0.02	0.20
PDL	-0.17	0.07	0.02	0.20	0.50**	-0.17	0.12	0.12	-0.42*	-0.42*	-0.10
LS	-0.10	0.07	0.07	0.19	0.03	0.17	0.22	0.22	0.02	-0.06	0.47*
WS	0.09	0.08	0.02	0.12	0.18	0.29	0.03	0.03	0.15	-0.13	0.39
WTH	0.00	-0.40*	-0.11	0.04	0.61***	0.33	0.22	0.22	0.37	-0.14	0.10
RSF	0.09	0.41*	-0.01	0.12	-0.41*	-0.01	-0.08	-0.07	-0.17	-0.08	0.20
PFH	0.46*	-0.18	-0.35	-0.11	0.17	0.23	0.06	0.10	0.07	-0.03	0.11
SFH	0.46*	-0.24	-0.38	0.17	0.14	0.19	0.12	0.05	0.08	-0.01	0.08
ALT	0.28	0.43*	-0.10	0.23	-0.57***	-0.10	-0.77	-0.08	0.49*	-0.13	0.18
RNF	0.14	0.46	-0.20	0.19	0.36	0.02	0.01	0.01	-0.41*	0.19	-0.04

LSH: Leaf shape, CW: Crescent width, AB: Arm breadth, AC: Arms color, FLU: Intensity of the flush, SP: Stipule pigmentation, CTP: Calyx tube pigmentation, PH: Petiole hairiness, SH: Stem hairiness, PDH: Peduncle hairiness, SSH: Seed shape, DSE: Date of seedling emergence, WDH: Winter development in height, SDH: Spring development in height, SDW: Spring development in width, WGS: Winter growth speed in width, SGS: Spring growth speed in width, FB: Flower bud, 1F: First flower appearance, FF: Full flowering, EF: End of flowering, DF, Duration of flowering, 1FH: First fruiting head appearance, FFH: Full formation of fruiting heads, NFP: Number of flowers per plant, PDL peduncle length, LS: Length of the seed, WS, The width of seed, WTH: Weight of 30 fruiting heads, RSF: Weight of seeds to the weight of the fruiting heads ratio, P/FH: number of pods per fruiting head, S/FH: Number of seeds per fruiting head, ALT: Altitude, RNF: Rainfull. Probability (signification): $p^* \leq 0.05$, $p^{**} \leq 0.01$, $p^{***} \leq 0.001$

Abdi *et al*., (2020) suggested the implication of morphological markers in environmental adaptation in this species, or the genes controlling them may be linked to other genes controlling adaptive traits. In this view, Francis & Millington (1965) and Kaur *et al*. (2017) reported that QTLs related to leaf marks, calyx pigmentation and isoflavonoids (formononetin, genistein and biochanin) were associated with the same chromosomal region on the genetic map of *T. subterraneum*.

Within the same genus (*Trifolium*), previous studies indicated a negative relationship between rainfall and the beginning of flowering in Algerian populations of *T. campestre*, *T. scabrum* and *T. tomentosum* (Issolah *et al.*, 1993; Issolah & Abdelguerfi, 1995, 2003; Issolah, 2006, 2018). The altitude of the origin environment of the populations influences positively the weight of thousand grains in *T. campestre*, *T. resupinatum*, *T. scabrum*, and also affects the number of pods per fruiting head and the length of fruiting heads in *T. scabrum* and *T. campestre* (Issolah *et al*., 1993; Issolah & Abdelguerfi, 1995, 2003; Issolah, 2006, 2018). In *T. resupinatum*, Issolah & Abdelguerfi (2002) showed that the seeds weight /fruiting heads weight ratio evolved with longitude.

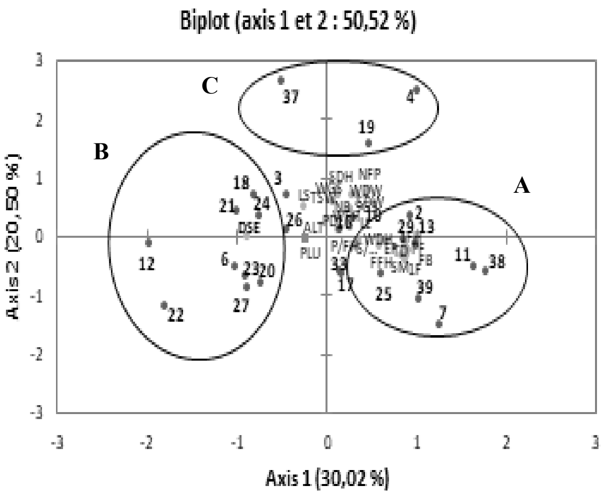

Fig.1. Principal component analysis (PCA) of quantitative morpho-phenological and biometrical characteristics of Algerian populations of *Trifolium subterraneum* L.

Algerian populations of *T. subterraneum*, a principal component analysis (PCA) was performed. Twenty-seven quantitative variables, including the two ecological factors of the natural habitats of the populations (altitude and rainfall), were considered. The plan 1-2 extracts 50.52% of the total variation (Fig. 1). Axis 1 in an axis of phenology and clearly determines the precocity of the populations (Fig. 1). It is positively correlated with phenological variables related to flowering and fruiting heads formation (FB, 1F, FF, EF, 1FH, SM). It is also negatively correlated with the seedling emergence date (DSE). For axis 2, which expresses the architecture of the plant and its production, the highest values located on the positive side of this axis are associated with variables related to the vegetative development (WDW, SDH, SDW, WGR, SGR) and those linked to its production (NFP, WTH, LS, TSW). The dispersion of the populations on the plan 1-2 of PCA (Fig. 1) indicates that they are distinguished into three main morphological groups. Axis 1 opposes the populations of the first group (A) (2/10, 7/10, 11/10, 13/10, 25/10, 29/10, 38/10, 39/10), which are late in flowering and fruiting heads formation, to the populations of the second group (B) (6/10, 12/10, 18/10, 20/10, 21/10, 22/10, 23/10, 24/10, 27/10), which are late in the seedling emergence and early in flowering and fruiting heads formation. Axis 2 distinguishes on its positive side the populations of group (C) (4/10, 19/10, 37/10), which are characterized by good vigour in terms of vegetative development (length and width), good growth speed, better flower production and heavier fruiting heads.

The results of the PCA reveal the existence of a remarkable morpho-phenological and biometrical variability within the Algerian populations of *T. subterraneum*, particularly in those coming from the Guelma region. This variability may contribute to the valorization of this species in different regions of Algeria. In a previous study conducted on the same Algerian populations of *T. subterraneum*, Issolah *et al*., (2015) also highlighted a diversity of edaphic, climatic and topographic parameters of the prospected natural habitats, in which the species seems well adapted. Furthermore, the karyological study conducted by Bouziane *et al*., (2019) on the same Algerian populations of *T. subterraneum*, permitted to observe two chromosomal formulas (2n=16; 2n=18) in the same individual and within the same population, especially in populations originating from high altitude regions. According to the same authors, the ecological conditions of the original habitat of the populations would have an effect on genetic changes and karyological structure, in particular the altitude (Bouziane *et al.*, 2019).

Within the same family (Fabaceae), similar results were obtained in some species belonging to the genera *Trifolium* (Issolah & Abdelguerfi, 1999 b; Issolah, 2018) and *Hedysarum* (Issolah *et al*., 2006; Benhezia *et al*., 2013).

Conclusion

This study examines the morphological structuring and behaviour of twenty-six (26) populations of the species *Trifolium subterraneum* L., originating from different regions of Northeast Algeria. Thus, most of the studied characteristics indicated very highly significant differences, denoting a wide intra-specific and interpopulation phenotypic variability. The characteristics related to the biometry and the phenology of the plant discriminate better the populations compared to the qualitative morphological ones, whose variability is relatively weak for some traits. Significant relationships were found between some quantitative traits and qualitative markers. The latter seem to be strongly linked to the characteristics related to the reproduction and the production of the plant. The significant correlations established between some quantitative and qualitative characteristics and the factors of the origin environment of the populations (Altitude and Rainfall), indicate that the altitude seems to play a more important role on the observed variation within Algerian populations of *T. subterraneum*. The characteristics related to the precocity, the vegetative development of the plant and the production, permitted to differentiate three groups of populations. The Principal Component Analysis (PCA) indicates a wide phenotypic variability, particularly in the populations coming from the region of Guelma. The results also showed that certain quantitative (size of floral peduncles and size of the internodes) and qualitative characteristics (pigmentation of the calyx tube, seed shape and degree of burial), correspond much more to the traits of the subspecies *subterraneum*. This allowed us to suggest the ssp. *subterraneum*, as an infra specific category to which belong the Algerian populations of *T. subteraneum*, originating from the considered Northeast regions. These new data on the morphology of *T. subterraneum* would be useful in order to clarify the classification of the natural populations corresponding of this species in Algeria and to conserve and use them in a breeding and sustainable development programme.

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