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Agro-morphological homogeneity of lucerne (*Medicago sativa* L. *subsp. sativa*) half-sib progenies bred for outside oases conditions of southern Tunisia

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Abstract - Thirty-nine lucerne half-sib progenies were been evaluated in this research. They derived from a polycross of native and exotic germplasm collections used as genetic material to hold a breeding program focusing on the development of new synthetic variety more adapted to outside oases arid conditions of southern Tunisia. The objectives of this work were to explore the variability among and within these progenies on basis of six agro-morphological traits, to evaluate their relating homogeneities and to classify them into similar groups after identifying the main traits contributing to the overall variability using multivariate PCA and hierarchical cluster analysis. Obtained results revealed a noticeable variability between and within progenies regarding the investigated traits. PCA showed that the first PC, which is the most important component, accounted for 34.45% of the total variability and was associated with FMY and DMY while the second PC accounted for 25.72% of the variability mainly resulted from the variation in ClrF, LCL and WCL. The cluster analysis performed on the first two PCs (60.18% of total variability) grouped the studied Lucerne progenies into three clusters. Analogically, with the superposition of each progeny with its assessed variables mean values and their relative CV%, it revealed that the progenies belonging to the first cluster (L4, A17, ABT21, L23, ABT32, E34, L39, S47, ABT52, A56, S71 and A73) presented the highest means values of the investigated traits and the least CV%. These progenies can be considered as the most homogenous material and the most appropriate to be selected to pursue the breeding scheme.

Keywords: alfalfa, agro-morphological, evaluation, coefficient of variation, multivariate analysis, arid conditions.

1. Introduction

Medicago sativa L. subsp. *sativa*, commonly known as cultivated alfalfa or lucerne, is a perennial herbaceous auto-tetraploid (2n = 4x = 32) forage legume (Muller *et al.* 2003, Annicchiarico *et al.* 2015) cropped in more than 80 countries accounting for 2.5% of all crops grown in the world (Živković *et al.* 2012). It has a broad reputation to be among the most appreciated fodder crops thanks to its wide agronomic, economic and environmental assets. In fact, it owes a high nutritional quality by way of its protein content and energy intake. Besides, it contributes to the economic sustainability of livestock-crop systems. Furthermore, it is owing a good adaptability to a multitude of environmental ranges thanks to its important genetic diversity (Deshpande *et al.* 2002). Indeed, lucerne populations are subjected to a high phenotypic and genetic heterogeneity at inter and intra-genotype levels such it is a cross-pollinated species with expressed self-incompatibility (Portablia *et al.* 1982). Consequently, evaluation, characterization and screening of genetic resources are considered priorities in lucerne breeding; such information is essential in selecting gene pool for breeding activities. Morphological traits concerning growth habit, shoots, leaves, flowers, and seeds are the direct expression of the genetic construction of a genotype. For that, plant morphometry has been used in different systems of classification (Smith *et al.* 1991, Loumerem *et al*. 2007, Moawed 2016). Fundamentally, the development of new improved plant varieties must fulfill three crucial criteria ; Distinctness, Homogeneity and Stability (DHS) before the registration procedure (UPOV, 1988).

In southern Tunisia, lucerne has been cultivated since many years. It is currently grown on more than 12 410 ha. Approximately 75% of oasis surfaces reserved for alfalfa (9720 ha) are in the Southern Tunisia (Abid *et al.* 2016). Native ecotypes '*Gabssia*' are a widely diversified genetic inheritance. In

spite of its richness, this inheritance is threatened by the genetic erosion phenomenon. Above and beyond, According to Loumerem *et al.* (2007) and Ben Abderrahim *et al.* (2009), these populations were well adapted for oases conditions, but outside oases (irrigated areas) they are less adapted. In this study, we have evaluated and characterized poly-cross half-sib progenies selected among genetic material used in a breeding program held in the Dry lands and Oasis Cropping Laboratory of the Arid Land Institute of Médenine, Tunisia (Loumerem *et al.* 2007, Tlahig and Loumerem 2014). The thirty-nine superior progenies were used for agro-morphological characterization. To make comparison of the variability of data groups with quite different means, the coefficient of variation (CV %) was used. The CV% expresses the standard deviation (δ) as a percentage of the mean and is independent of measurement units. In biological experiments, a coefficient of variation of 10% or less is generally desired (Poehlman *et al.* 1995). Multivariate analysis is a very useful method because it reveals the relationships and correlation among variables studies. This type of analysis applied to studies of germplasm collection allows a better understanding of the structure of the collection, identification of more relevant variables, detection of the relationships among genetic material, as well as identification of possible groups to pursue breeding schemes (Martines-Calvo *et al.* 2008).

The purposes of this research are to (i) assess the variability among and within the studied lucerne progenies on basis of each of agro-morphological traits, (ii) test the within-progenies homogeneity regarding these traits, (iii) identify the traits which were the main source of the variability and (iv) classify progenies using multivariate cluster analysis referring to their homogeneity coefficients.

2. Material and Methods

2.1. Plant material and experimental design

2.1.1. Origin of studied plant material

Twenty alfalfa '*Gabssia*' accessions had been collected from different oases of southern Tunisia under PERMED project missions. A detailed characterization of the accessions was given by Loumerem *et al.* (2007).This germplasm was employed to develop progenies including some germplasm derived of the foreign varieties (*Sardi10, Ameristand801S, ABT805* and *Siciliano Ecotipo*) considering their excellent response to the cropping conditions in the oases of southern Tunisia compared to the local populations '*Gabssia*' and other North-African cultivars. Hundred genotypes were used like parents in this breeding program. The breeding design to select synthetic varieties for arid regions of Tunisia was detailed by Loumerem *et al.* (2008) based on the evaluation of the polycross progenies performances (Tlahig and Loumerem 2014).

The thirty-nine superior half-sib progenies were used to carry out this research. These progenies are described in the table 1 below.

Table 1. Origin of studied lucerne half-sib progenies.

Progenies	Half-sib family	Type	Geographic Origin	Selection criteria
L4, L16, L18, L19, L20, L23, L33, L39, L51, L53, L55, L57, L58, L61, L64, L67, L68	*Gabssia*	landrace	Coastal oasis, Tunisia	Oasis conditions (Loumerem *et al.* 2008)
A11, A17, A49, A56, A73	*Ameristand801S*	variety	USA	Salt tolerance (Pecetti *et al.* 2013)
ABT21, ABT32, ABT52, ABT65, ABT70	*ABT805*	variety	USA	Grazing tolerance (Bouton *et al.* 2001)
E34, E40, E42, E60, E69	*Ecotipo siciliano*	landrace	Sicily, Italy	Rain-fed conditions (Pecetti *et al.* 2008)
S3, S43, S44, S47, S66, S71	*Sardi10*	variety	Australia	High winter activity (Pembleton *et al.* 2010)

2.1.2. Experimental design and investigated parameters

Each of the studied progenies was sown along simple rows. They were arranged in random complete blocks design with three replications; with twelve plants per row on each block. The plants were spaced of 40 cm on the rows of culture with a row-space of 40 cm. During the essay period, 13 cuttings were undertaken in the range of one-tenth bloom in spring and summer to pre-bud stage in winter and autumn. Evaluated traits are listed in Table 2.

Table 2. List of agro-morphological evaluated parameters (IBPGR 1984)

parameter	Code	unit	scores
Cumulated fresh matter yield	FMY	g	weight
Cumulated dry matter yield	DMY	g	weight
Length of central leaflet at blooming	LCL	cm	measurement
Width of central leaflet at blooming	WCL	cm	measurement
Shape of leaf	ShL	3-5-7	3= elongated 5= ovate 7= round
Color of flower	ClrF	1-2-3-4-5	1= white 2= yellow 3= light blue-purple 4= dark blue-purple 5= red-purple

2.2. Statistical analysis

Obtained results were statistically analyzed using SPSS 20.0 and *Xl*Stat 2014 softwares. Quantitative traits were subjected to analysis of variances (one-way ANOVA) to test the variability within and among progenies. Homogeneity of these progenies regarding each investigated trait were identified as expressed by their relative coefficients of variations (CV%). Qualitative traits were analyzed by sorting the progenies on basis of percentages of each trait variants. Principal component analysis (PCA) was performed on all traits to identify those were the main source of the variability and to explain the genetic diversity among progenies. As the traits were measured on different scales, the mean observation of each traits were standardized by matching their related CV% to eliminate scale differences. Hierarchical clustering was carried out on the coordinates of every progeny on the first and the second PCs whose explained 60.18% of total variability.

3. Results

3.1. Cumulated Fresh and dry matter yields

Analysis of variances (ANOVA, 5%) revealed that there were no significant differences among the studied lucerne progenies for both forage yield traits. Therefore, obtained mean FMY and DMY fluctuated respectively between minima of 9.34 $kg.m^{-1}$ and 2.10 $kg.m^{-1}$ recorded for S71 progeny while A11 produced highest FMY and DMY (15.81 and 3.63 $kg.m^{-1}$ respectively). Between-population variance was about 40.92% for the FMY and 42.15% for the DMY. Within-population variances of FMY accounted from 22.70% to 36.57% obtained for "*Ameristand801S*" and "*Sardi10*" respectively, while those of DMY were between 23.41% and 35.77% for the same populations. Calculated coefficients of variation (CV %) regarding FMY were from 5.52% within the progeny S3 of "Sardi10" population to 39.90% within the progeny A11 of "*Ameristand801S*". Similarly, Lowest CV% regarding DMY was between 2.09% and 39.01% within the same two progenies S3 and A11 respectively (Figure1).

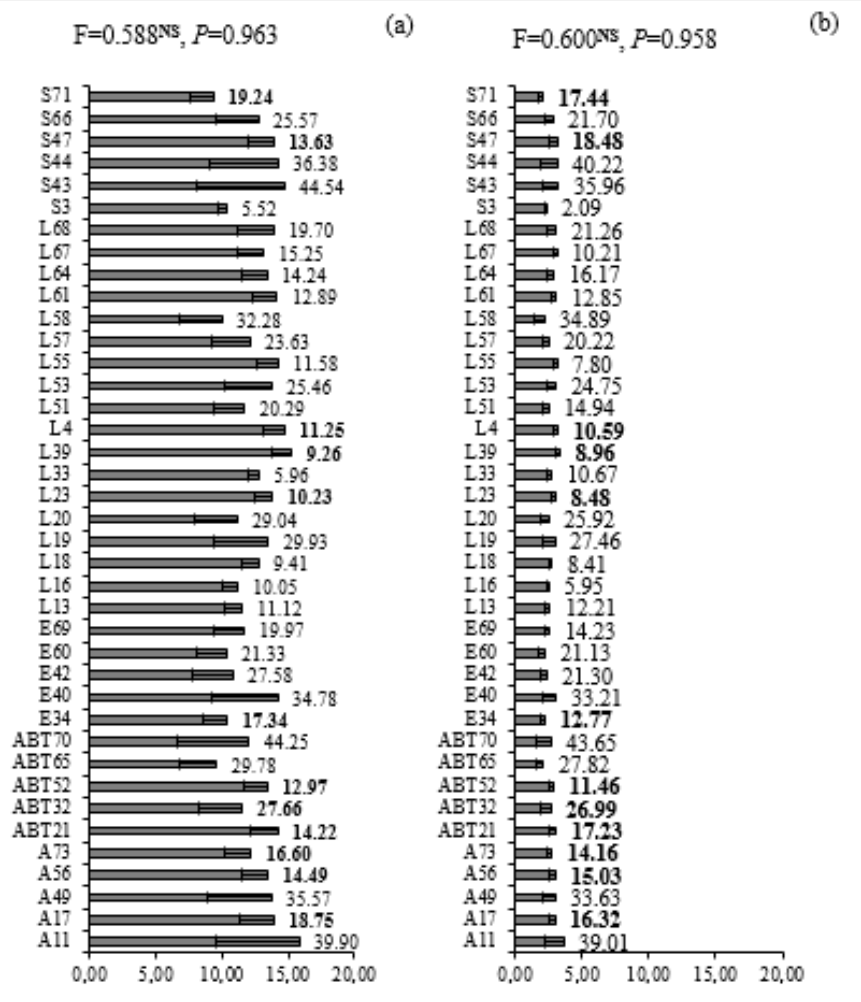

Figure 1. Cumulated fresh (a) and dry (b) matter yields homogeneity among 39 lucerne half-sib progenies bred for outside oasis arid conditions of southern Tunisia during 2010 cropping year. Histograms present means with negative SD error-bar, followed by CV% values. One-way ANOVA results are mentioned in the upper zone of the graphic as F value (0.05) followed by *P* values significance: ^{NS} $P \geq 0.05$,* $P < 0.05$.

3.2. Length and width of central leaflets at blooming

ANOVA showed significant differences only for the length of central leaflet at blooming (LCL) under the progeny effect. Widths of central leaflets at blooming (WCL) did not differ significantly among studied lucerne progenies. Highest LCL average was 3.08 cm obtained for the progeny A73 while lowest one was 2.22 cm obtained for L16. Although, WCL averages were from 1.08 cm for the progeny E42 to 1.64 cm for ABT52. Between-populations variances of lengths and widths of central leaflets at blooming were about 27.92% and 34.14% respectively. Somewhat, within-population variances of LCL were flanked by 14.28% inside "*Sardi10*" population and 25.64% within "*Ameristand801S*" ; while those of WCL were between 8.39% in "*Ameristand801S*" and 26.21% inside "*ABT805*".

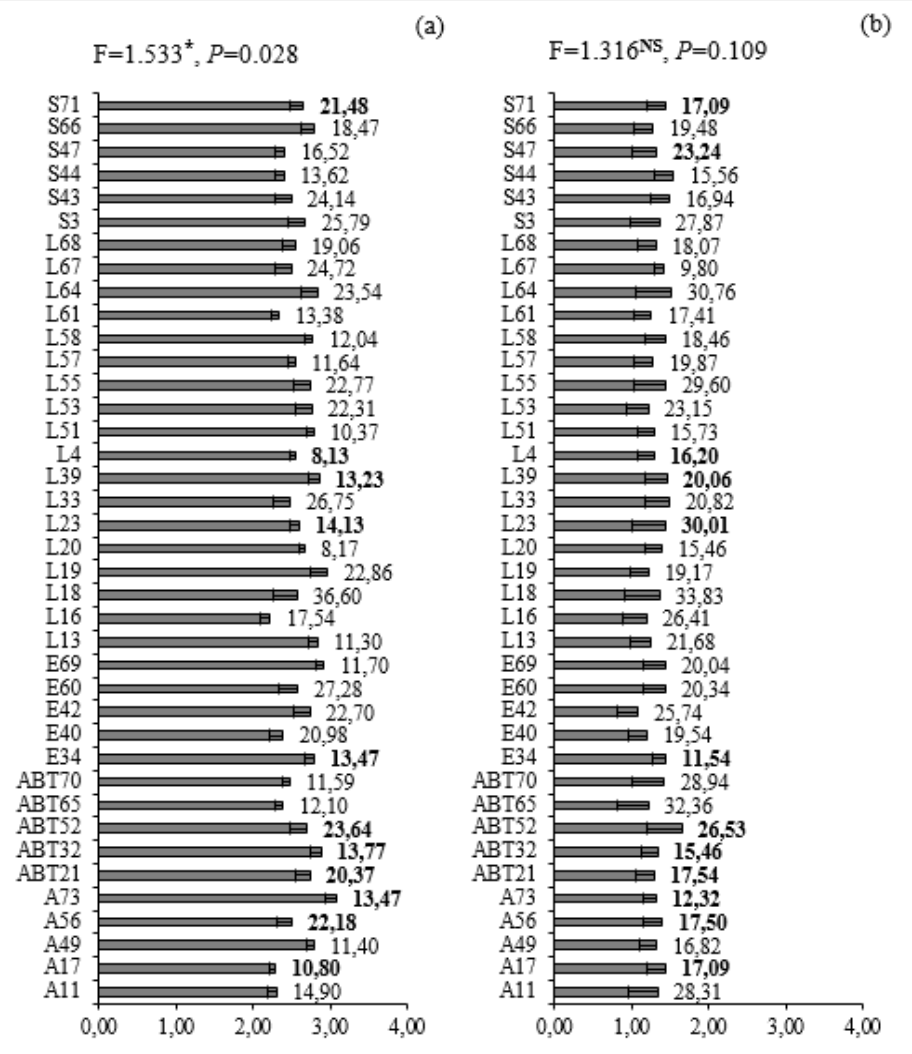

Figure 2. Homogeneity of length (a) and width (b) of central leaflet at blooming among 39 lucerne half-sib progenies bred for outside oasis arid conditions of southern Tunisia during 2010 cropping year. Histograms present means with negative SD error-bar, followed by CV% values. One-way ANOVA results are mentioned in the upper zone of the graphic as F value (0.05) followed by *P* values significance ^{NS} $P \geq 0.05$, * $P < 0.05$

The most homogenous progeny regarding LCL was L4 owing a CV%=8.13 while the most heterogeneous was S43 having a CV%=24.14%. By the other hand, lowest CV% was around 9.80% within the progeny L67 and the highest one was over 33.30% within the progeny L18.

3.3. Shape of leaf

According to the forage legumes descriptor (IBPGR, 1984), the shape of lucerne's leaf can be either elongated, ovate or round. Concerning the studied progenies, 60% of plants had ovate leaves; the remaining ones owed an elongated shape of leaf. Depending on their degree of homogeneity, expressed as percent of each observed shape of leaf, the studied progenies were separated into eight groups.

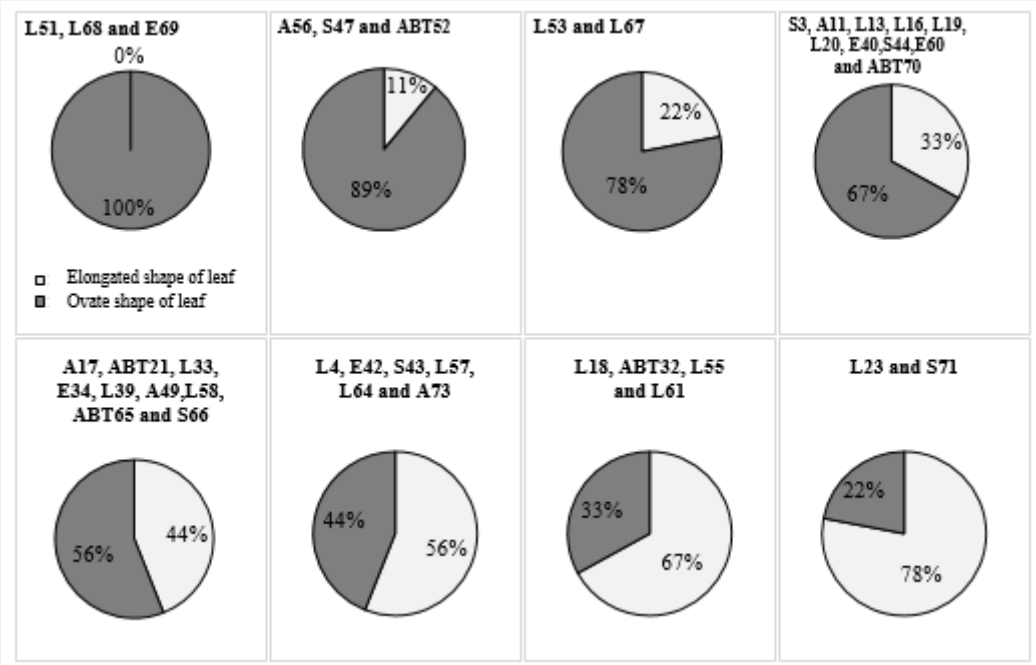

Figure 3. Classification of 39 lucerne half-sib progenies bred for outside oasis arid conditions of southern Tunisia during 2010 cropping year regarding the shape of leaf trait (n=24).

Five groups included progenies whose plants had formed mostly ovate leaves. The progenies of the other groups were mostly with elongated shape of leaf. Among the studied lucerne progenies, we checked that progenies L51, L68 and E62 were the most homogenous with 100% of plants having an ovate shape of leaf; followed by progenies A56, S47 and ABT52 with 89% of ovate leaves and 11% of elongated ones.

3.4. Color of flowerings

According to the forage legumes descriptor (IBPGR 1984), flowering of lucerne can be evaluated by percentage of each color. By the way, the lucerne flowering color diversity includes five tints: white, yellow, light blue- violet, dark blue-violet and red-violet. Obtained results showed that the flowerings of the studied progenies are either dark blue-violet or light blue-violet. Analysis shows that 67% among plants of all progenies had light blue-violet flowers. The remaining progenies' flowerings are dark blue-violet. Nine groups of similar progenies, depending on the homogeneity in color of flowerings were distinguished. The most homogenous groups were the first and the last ones; the first included progenies L4, L16, L18, ABT21, L39, E40, S47, A49, E60 and L61 whose flowers are 100% light blue-violet; however, progenies A11, L53, S66 and A73 belonging to the ninth group had 100% of dark blue-violet flowers. The following homogenous progenies are ABT52 which represent a group on itself; it contains 89% of plants with dark blue violet flowers, and in the same range of homogeneity, the group including progenies L13, L19, L20, E34, E42, S43, S44, L55, A56 and E69 had 89% of plants with light blue violet flowers.

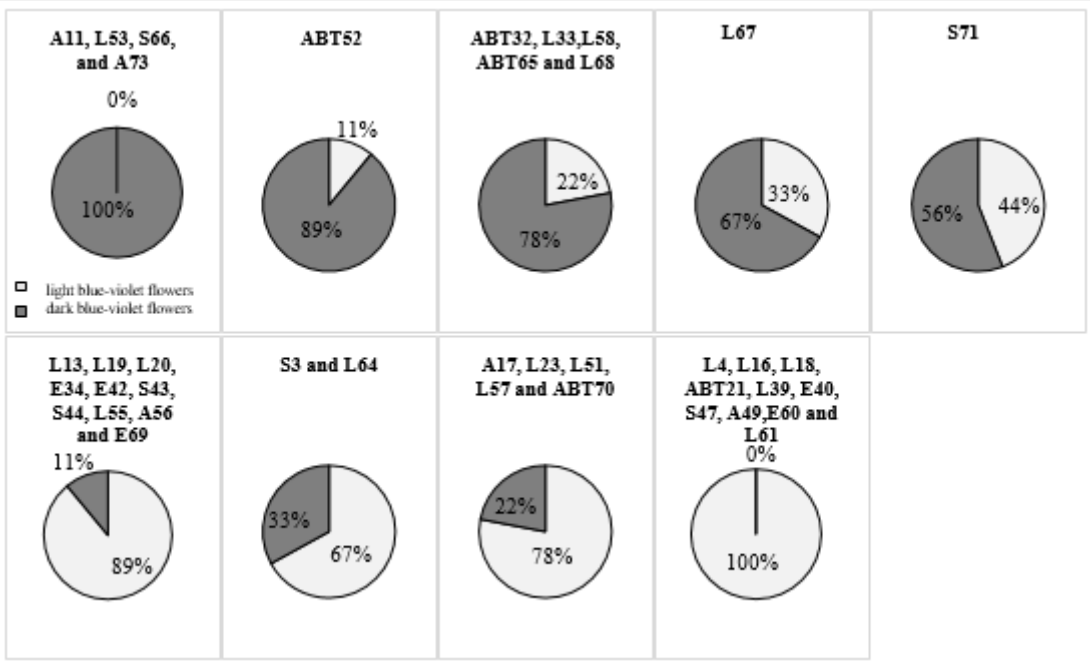

Figure 4. Classification of 39 lucerne half-sib progenies bred for outside oasis arid conditions of southern Tunisia during 2010 cropping year regarding the color of flowering trait (n=24).

4. Discussion

In most classical Lucerne breeding programs, the value of the subjected germplasm has to be defined upon mean values and variability of the most representative agro-morphological traits. Phenotypic characterization is among crucial steps toward the germplasm clustering (Smith *et al*. 1991, Ghafoor *et al*. 2003). The choice of appropriate superior genetic material candidate to the achievement of the breeding program needs a compromise between higher mean values and within-genotype homogeneity upon the assessed traits.

Multivariate analysis and clustering of studied Lucerne progenies

Multivariate analysis is a very valuable process revealing the relationships and correlation among variables studies. This kind of analysis has been applied to studies of germplasm collection and allowed an enhanced understanding of the structure of the genetic pool, identification of more relevant variables, detection of the relationships among genotypes, as well as identification of possible groups (Tucak *et al*. 2009). Principal Component Analysis (PCA) has been broadly used in the studies of variability in germplasm collections of many species (Julier *et al.* 1995, Bennett 2000, Veasey *et al*. 2001, Naghavi and Jahansouz 2005, Zakova and Benkova 2006, Martines-Calvo *et al.* 2008).

The PCA analysis was applied to identify the traits that were the main source of the variability and to explain the genetic diversity in germplasm collections. The first three principal components (PCs) gave Eigen values greater than 1.0 and explained 77.35% of the total variability among the progenies for all investigated traits (Figure 3). Jenczewski *et al.* (1999) analyzed gene flow between wild and cultivated alfalfa populations using thirteen quantitative traits and found that the first four PCs contributed 75% of the entire variability among the twenty populations. Tucak *et al.* (2009) evaluated thirteen morphological traits on twenty-seven alfalfa populations/cultivars and found that the first four PCs contributed 89.02% of the entire variability. The first PC, which is the most important component, accounted for 34.45% of the total variability and was associated with FMY and DMY. Similar result was reported by Annicchiarico (2006) found that the first PC included only 35% of the total variation. Prosperi *et al.* (2006), who studied morphological and agronomical diversity of wild genetic resources of alfalfa, detected that the first PC explained 56.4% of the total variability in the measured traits and was associated with biomass production, which is congruent with our results.

The second PC accounted for 25.72% of the variability. This portion of variation mainly resulted from the variation in ClrF, LCL and WCL. Comparably, Tucak *et al.* (2009) found that the second PC

accounted for 16.24% of the variability. This portion of variation mainly resulted from the variation in number of stems, shape of leaf and width of central leaflet.

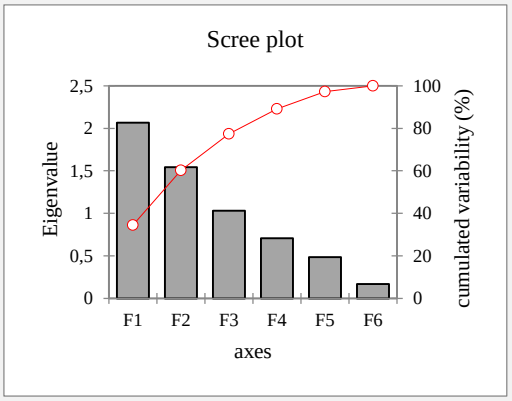

Figure 5. Eigenvalues and proportion of total variability explained by the six PCs.

Table3. Contribution of the investigated traits into the six principal components

	F1	F2	F3	F4	F5	F6
FMY	**0,5774**	0,3675	0,0490	0,1226	0,1005	0,7100
DMY	**0,5603**	0,3525	0,2532	0,0208	0,1730	-0,6836
LCL	-0,4453	**0,4337**	-0,0736	0,0201	0,7791	0,0291
WCL	-0,2535	**0,3814**	0,5820	-0,5951	-0,2912	0,1126
ShL	-0,0894	-0,3562	0,7664	0,4772	0,2037	0,0930
ClrF	-0,2865	**0,5295**	-0,0460	0,6343	-0,4762	-0,0800

4.1. Classification of lucerne progenies regarding their Multivariate homogeneity

The cluster analysis performed on the first two PCs (60.18% of total variability) grouped the studied Lucerne progenies into three clusters (Figure 6). The first cluster gathered progenies L4, A17, ABT21, L23, ABT32, E34, L39, S47, ABT52, A56, S71 and A73. The second cluster separated the progeny L67 from all others while the last cluster included the remaining progenies.

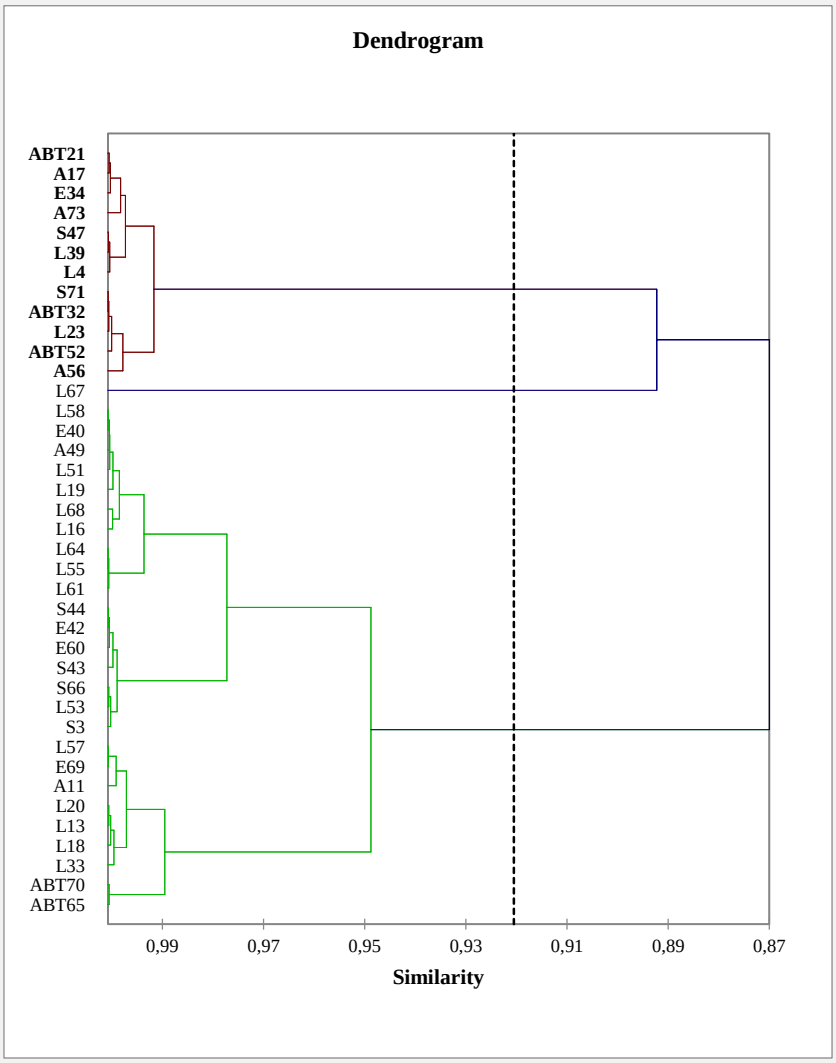

Figure 6. Multivariate hierarchical cluster of 39 lucerne half-sib progenies bred for outside oasis arid conditions of southern Tunisia during 2010 cropping year regarding their CV% related to assessed agro-morphological traits.

Analogically with the superposition of each progeny with its assessed variables mean values and their relative CV%, it revealed that the first cluster included the progenies having the highest means values of the investigated traits and the least CV% indicating that they can be considered as the most homogenous material and the most appropriate to be selected to pursue the breeding scheme.

5. Conclusion

In terms of this work, an assessment of agro-morphological traits was valuable to select twelve superior lucerne progenies to achieve the breeding program focusing on the development of new synthetic variety more adapted to outside oases conditions of Tunisian arid regions. for a further work, the test of other agronomic qualities such nutritive value, tolerance to abiotic stresses those are among the most serious cropping limits have to be investigated to gather a wider identification of this studied genetic pool.

Acknowledgements

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