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A COMPARISON OF PERFORMANCE AMONG EXOTIC AND LOCAL ALFALFA (*MEDICAGO SATIVA* L.) ECOTYPES UNDER TUNISIAN CONDITIONS

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ABSTRACT

The objective of this paper work was to compare performance among foreign and local alfalfa ecotypes under Tunisian conditions, in view of improvement programme. For this goal, 20 alfalfa farm ecotypes were collected from Tunisian oases and 16 exotic cultivars were provided by PERMED project. The evaluation of agro-morphological traits on all the populations was performed during two years (2006-2008) in experimental field of Arid Regions Institute, Gabes (33.90°N; 10.08°E), of Tunisia. Except width of central leaflet, all agro-morphological traits were significantly different among populations (P<0.05). Higher dry matter percentage, stem length, inflorescences.plant^{-1} and pods.plant^{-1} were mainly associated with local populations. Exotic germplasm had advantage in fresh weight, stem number and weakness in reproductive traits. The two plans of the projection of the first, the second and the third components in the Principal Component Analysis showed an excellent separation between the local and foreign alfalfa cultivars. This shows that the variables analysed are able to explain the differences observed between the native and foreign alfalfa populations. The first axis showed that the exotic populations were distinguished by highest fresh weight yield and leaves number, strong length of central leaflet, than the native ones. The correlation study showed the possibility of selection of synthetic variety combining higher green matter yield and good digestibility or high dry and seed yield in one genotype.

Key words: Lucerne, assessing, populations, native, indigenous, arid-climate.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is one of the most important forage crops in the world, and is believed to have its origin from Caucazian region (Michaud et al., 1988). It is autotetraploid ($2n=4x=32$) (McCoy and Bingham, 1988), cross-pollinated (allogamous) and seed propagated. In North Africa regions, lucerne germplasm is considered an elite plant material such as in Tunisia and Morocco where the cultivar development has not progressed over the last few years for diverse reasons (PERMED, 2004). Currently, alfalfa genotypes are organized in different populations where each one is adapted to the local climatic conditions. Populations from North Africa are mainly landraces. Traditionally, each farmer produces his own seeds, but seed exchanges between farmers are frequent (Julier et al., 2010). Tunisia is a large forage legumes cultivating country. These species are mainly cultivated in the oases, where farmers use both native and foreign germplasm. Since 1960, one cultivar called "Gabssia" has been used in these regions (Le Houérou, 1965) and it is actually genetically polluted. Elghazzah and Chalbi (1995) reported that the introduction of foreign cultivars for higher yield induced a genetic pollution in local diversity.

Evaluating the genetic variability of alfalfa cultivars is important to identify the maximally diverse parental genotypes (Falahati-Anbaran et al., 2006) especially for agro-morphological traits. In addition, the analysis of genetic diversity in forage species such as alfalfa is an important step for seed purity analysis and germplasm management (Falahati-Anbaran et al., 2006). Currently, the available seeds are auto-produced from local populations or bought from commercial populations (varieties or landraces). The erosion of genetic diversity in most of owner cultivated species underlines the need to describe genetic variation through collection material which could be used in

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breeding programs. Although the genetic variation among populations has frequently been tested on many varieties and landraces, the comparison among native and exotic genotypes has not been considered to the best of our knowledge, particularly in regions which import alfalfa seeds such as Tunisia, Morocco, Algeria. In this paper work, our purpose is to evaluate the inter-population alfalfa diversity among local and foreign germplasm, cultivated in Tunisia using morphological, agronomic, growth and reproductive traits to select new (synthetic) varieties.

MATERIAL AND METHODS

Germplasm collection

Thirty six populations were studied (Table 1). Native alfalfa landraces seeds were collected from Gabes, Tozeur and Kebili (Figure 1) districts in south of Tunisia. Sixteen exotic landraces and varieties were provided by the project "*Improvement of native perennial forage plants for sustainability of Mediterranean farming systems*" (PERMED, 2004).

Table 1. Name, origin, and main characteristics of studied alfalfa populations (*Medicago sativa* L.)

Codes	Name	Country	Type of population	Type or user	Region
P1	Kattana	Tunisia	Landrace	Irrigated oasis	Gabès
P2	Chenchou	Tunisia	Landrace	Irrigated oasis	Gabès
P3	Chenini-1	Tunisia	Landrace	Irrigated oasis	Gabès
P4	Chenini-2	Tunisia	Landrace	Irrigated oasis	Gabès
P5	Chenini-3	Tunisia	Landrace	Irrigated oasis	Gabès
P6	Tboulbou	Tunisia	Landrace	Irrigated oasis	Gabès
P7	Metouia	Tunisia	Landrace	Irrigated oasis	Gabès
P8	Ghannouch	Tunisia	Landrace	Irrigated oasis	Gabès
P9	Zerkine	Tunisia	Landrace	Irrigated oasis	Gabès
P10	Essdada	Tunisia	Landrace	Irrigated oasis	Tozeur
P11	Bouhlel	Tunisia	Landrace	Irrigated oasis	Tozeur
P12	Dgach	Tunisia	Landrace	Irrigated oasis	Tozeur
P13	Hammajerid	Tunisia	Landrace	Irrigated oasis	Tozeur
P14	Zaafrane	Tunisia	Landrace	Irrigated oasis	Kébili
P15	Nouael	Tunisia	Landrace	Irrigated oasis	Kébili
P16	Jersine	Tunisia	Landrace	Irrigated oasis	Kébili
P17	Elgolaa	Tunisia	Landrace	Irrigated oasis	Kébili
P18	Limaguess	Tunisia	Landrace	Irrigated oasis	Kébili
P19	Douz	Tunisia	Landrace	Irrigated oasis	Kébili
P20	Stiftimia	Tunisia	Landrace	Irrigated oasis	Kébili
P21	Sardi 10	Australia	Variety	Irrigated oasis	-
P22	Esicilia	Italy	Variety	Irrigated oasis	Sicily
P23	ABT	USA	Variety	Irrigated oasis	-
P24	Amerist	USA	Variety	Irrigated oasis	-
P25	Erfoud 1	Morocco	Landrace	Pre-saharian oasis	Erfoud
P26	Melissa	France	Variety	Pre-saharian oasis	-
P27	Siriver	Australia	Variety	Hunter river	-
P28	Rich 2	Morocco	Landrace	Pre-saharian oasis	Demnate
P29	Demnat 203	Morocco	Landrace	Valley	Draa valley
P30	Tamantit	Algeria	Landrace	Pre-saharian oasis	Addrar
P31	Gabès 2355	Tunisia	Variety	Irrigated oasis	Gabès (Coastal)
P32	Magali	France	Variety	Provence	-
P33	Prosementi	Italy	Variety	-	-
P34	Mamountanas	USA	Variety	-	-
P35	Coussouls	France	Variety	-	-
P36	Africaine	Morocco	Landrace	Irrigated oasis	-

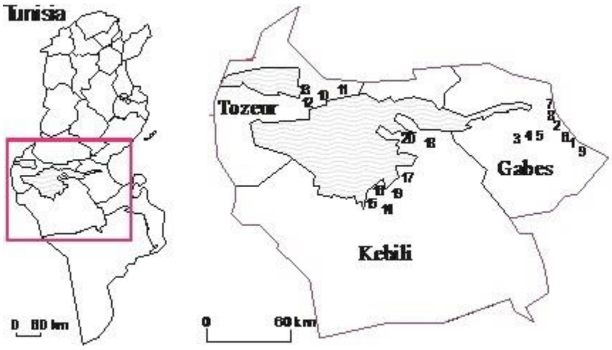

Figure 1. Origins of local populations from the three provinces of Gabes, Tozeur and Kebili in south of Tunisia. Numbers indicated population codes

Experimental design

The thirty six populations were installed and evaluated *ex situ* on sandy soil, at the experimental field of the Arid & Oasis cropping laboratory in the Arid Regions Institute-Gabès, Tunisia (33.90°N; 10.08°E). All parameters were determined in four replications for each population during two years. Edapho-climatic conditions of the experimental site were similar to oasis characteristics: the annual rainfall is 180 mm, the minimum mean temperature in winter is 12°C and the maximum mean temperature at summer is 28°C. The Randomized Complete Block Design with four replicates was used. The seeds were mechanically scarified to obtain a homogeneous germination (Crochemore et al., 1998). The experiment was started on September, 20th, 2006, in plots of 8 m^2, 4 m in length and 2 m in width. Seeds were planted using a drill at the seed rate of 40 kg ha^{-1}. Chemical fertilizer (32 kg ha^{-1} of nitrogen as ammonia and 70 kg ha^{-1} of P_2O_5 as biphosphate) was applied 2 weeks before seeding. Plants were surface-irrigated at regular intervals of 25 days (oasis frequency). Weeds were controlled with hand weeding when necessary.

Characteristics

The properties measured on all plots were: emergence time (ET; in days after seeding date), fresh yield (FW in g/m^2, above-ground parts in six harvests was weighted, in two years) and dry matter percentage (DW; % from 500g fresh matter dried at 80 °C at room temperature). Stem length (cm), stem number per plant, number of leaves per stem, length of central leaflet (LC in cm), width of central leaflet (WC in cm), foliar surface (FS in cm^2), and number of inflorescences per plant (Inf plant^{-1}) were determined on sample of 15 stems randomly collected from the centre of each plot at flowering time (two summers; 2006 and 2007). Pods number per plants (Pod plant^{-1}) was determined in august when the flowering period was completed.

Data analysis

The data collected for various traits were subjected to statistical analysis using Analysis of Variance test (ANOVA). The confidence interval was calculated at the threshold of 95%. Coefficients of variance (CV) were calculated to estimate the diversity for studied traits (Dong et al., 2001). The structure of variability among the populations based on all studies traits was analysis using a Principal Component Analysis (PCA) with the mean population values across the four replications. The inter-populations similarities were clustered into dendrogram groups using UPGMA command (Unweighted Pair-Group Method, Arithmetic Average). Pearson correlations between all studied characters were calculated. All analysis was performed using SPSS 16.0 software.

RESULTS

Variance analysis

Results of variance analysis of the 36 populations showed highly significant differences among populations for all characters (P<0.001, Table 2); except for width of central leaflet (WC). Diversity in each character is revealed using the Coefficient of Variation indices (Table 2). Number of pod per plant and Dry matter percentage traits represented the highest and lowest diversity with 14.87 and 2.00%, respectively. As shown by Figure 2, the central leaflet was longer for exotic than for local ecotypes and mean values were respectively 1.7 and 1.4 cm. Similarly, the number of stems and leaves were higher for exotic populations. The central leaflet (LC), foliar surface (FS), stem length and the seed emergence were, however, greater in native populations than in exotic foreign ones. For fresh yield, foreign populations were more productive (FW ranged from 1304.9 to 1558.9 g m^{-2}) than local ones (FW ranged from 740.6 to 967.9 g m^{-2}). In contrast, results showed higher percentages of dry matter in local ecotypes; values ranged from 28.9 to 29.1% in local populations and from 23 to 23.25% in exotic ones. Considering reproductive traits, native genotypes were more productive than foreign ones with an average of 100.2 for number of inflorescences and 272.3 pods per plant, respectively. For the foreign germplasm, the number of inflorescence and pods per plant were respectively 36.5 and 39.1.

Table 2. Analysis of variance for inter-population characteristics

Characters	Df	F	P%	CV%
FW	35	3.853	<0.0001	7.25
DW	35	214.146	<0.0001	2.00
Stem length	35	22.538	<0.0001	2.26
Stem branches	35	23.898	<0.0001	2.36
LC	35	5.563	<0.0001	3.76
WC	35	1.377	0.108	4.31
FS	35	18.290	<0.0001	3.63
leaves Number	35	65.565	<0.0001	4.41
Emergence	35	30.722	<0.0001	5.60
Inf $plant^{-1}$	35	2.699E3	<0.0001	10.14
Pod $plant^{-1}$	35	2.550E4	<0.0001	14.87

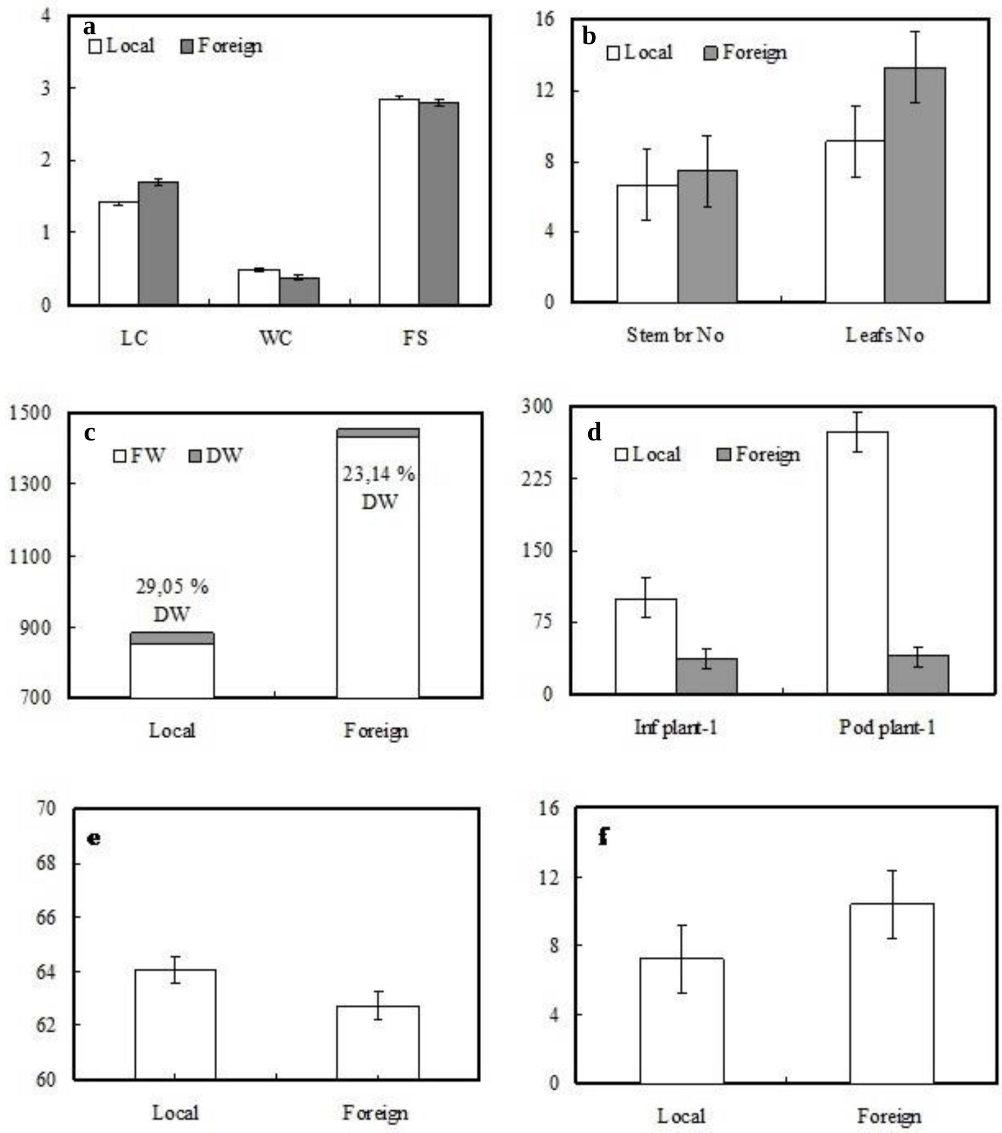

Figure 2. Comparison between local ecotypes and foreign ones of alfalfa cultivated in Mediterranean region. a: Length of central leaflet (LC in cm), Width of central leaflet (WC in cm) and foliar surface (FS in cm^2), b: Stem branch number and leaves number, c: Fresh weight and Dry matter (FW in g m^2, DW in %), d: Inflorescence and pod per plant e: Stem length (cm), and f: Emergence in days after sowing date.

Multivariate analysis

Principal component analysis

The PC analysis grouped populations on the basis of all traits. The first three components account for about 67.63 % of total variance. The first (PC1), the second (PC2) and the third (PC3) accounted for 41.17%, 15.6 % and 10.7 % of the total variation. Figure 3 displays the scores plot of the first three principal components (PC). Table 3 shows the contribution of the different characteristics to first three axis of the PCA.

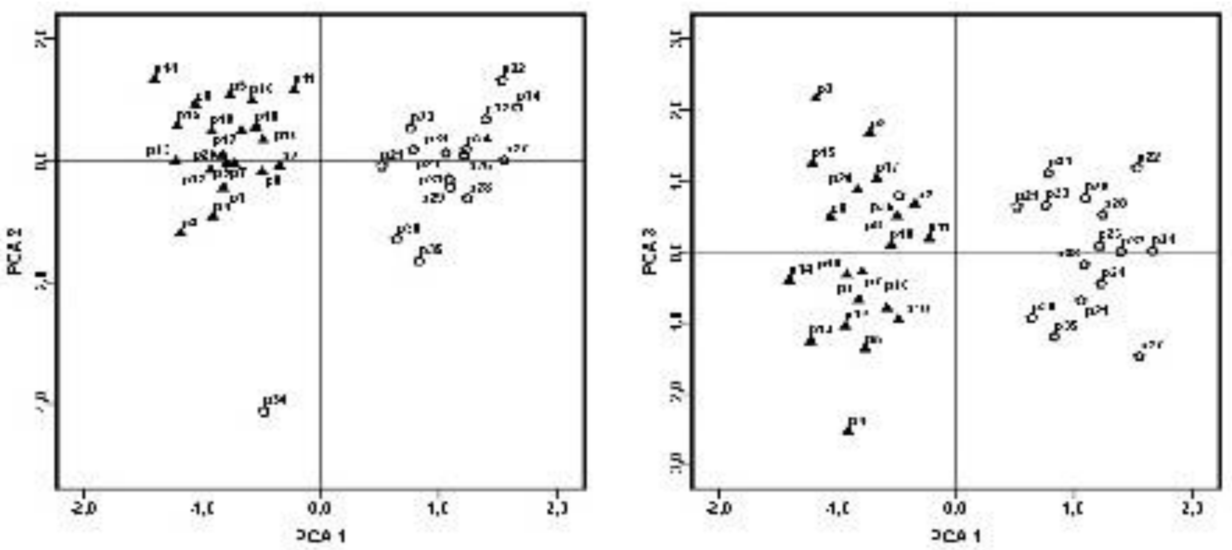

Figure 3. PCA diagrams for the alfalfa populations show the distribution of populations on the first two axes (PCA 1/PCA2), and on the first and third axes (PCA1/PCA3). Symbols: ▲: Native populations; ⌂: Foreign populations; px: population code.

The first component was positively correlated to the fresh weight; the length of central leaflet and the number of sheets (0.76, 0.66 and 0.85 respectively). Contrary, the reproduction traits (inflorescences and pod production) and the dry matter percentage were negatively correlated (-0.74, -0.80 and -0.91 respectively) to the PC1. However, the second component was positively correlated to the growth traits (stem length, branches number and foliar surface; 0.55). For the third axis, Table 3 shows that it was positively correlated (0.67) to the foliar surface and negatively correlated (-0.58) to the stem length. The two plans of the projection of the first, the second and the third components (Figure 3) show the excellent separation between the local and foreign alfalfa cultivars. This shows that the variables analysed were able to explain the differences observed between the native and foreign alfalfa populations. Compared to the native populations, the first axis showed that the foreign ones were distinguished by highest fresh weight production and leaves number, high length of central leaflet, low values of dry matter percentage and reproductive traits. Results related to the second axis separated populations according to highest stem length and number and to highest foliar surface.

Table 3. Correlations contribution of each character in the three first axes

Characters	PC1	PC2	PC3
% of variation	41.17	15.6	10.7
FW	0.762	0.112	-0.072
DW	-0.913	0.171	-0.013
Stem length	0.251	0.559	-0.585
Stem br. per plant	0.485	0.545	0.041
LC	0.661	0.482	0.035
WC	-0.481	0.241	0.477
FS	0.128	0.565	0.678
Leafs number	0.851	0.159	-0.114
Emergence	0.464	-0.347	0.061
Inf plant^{-1}	-0.740	0.456	-0141
Pod plant^{-1}	-0.803	0.305	-0.316

Local and foreign populations are interfered in this separated group, such as the landraces from Gabes-Tunisia (Cheneni-3 and Teboulbou), Tozeur-Tunisia (Essdada and Bouhlel) and Kebili-Tunisia (Limaguess and Douz) provinces and the introduced ones such as Italy (Sicily), USA (ABT and Mamoutanas) and France (Magali) varieties. The third axis separated the populations which presented low growth and large leaves. This group consisted in the North African landraces such as Tunisian (Cheneni 1, Zerkine, Elgolaa and Stiftimia) and Moroccan (Erfoud 1, Demnat 203 and Rich 2) ones.

Clustering analysis

As shown by Figure 4, clustering analysis based on the agro-morphological traits was performed as similarity dendrogram and all populations were delineated in eight clusters. Cluster one contained ten local populations, two of which shared 90% similarity of agro-morphological traits. Clusters two and three contained three and two local populations, respectively (similarity from 92 to 95). Cluster four contained 7 populations, five natives and 2 foreign, the five local populations showing similar phenotypic traits (96% similarity) with population P32 (Magali-France). Clusters 5, 6, 7 and 8 consisted of 3, 4, 3 and 4 exotic populations, respectively (similarity from 91 to 97%).

Correlations between all agro-morphological traits

There were significant correlations among most of pair-wise traits. Significantly negative correlation was found between fresh weight and dry matter (-0.73).

Correlation between fresh matter yield and leaves number was highly positive with coefficient of 0.66 (Table 4). Dry matter percentage was highly correlated to reproductive traits (number of inflorescence per plant: 0.72 and number of pods per inflorescence: 0.77).

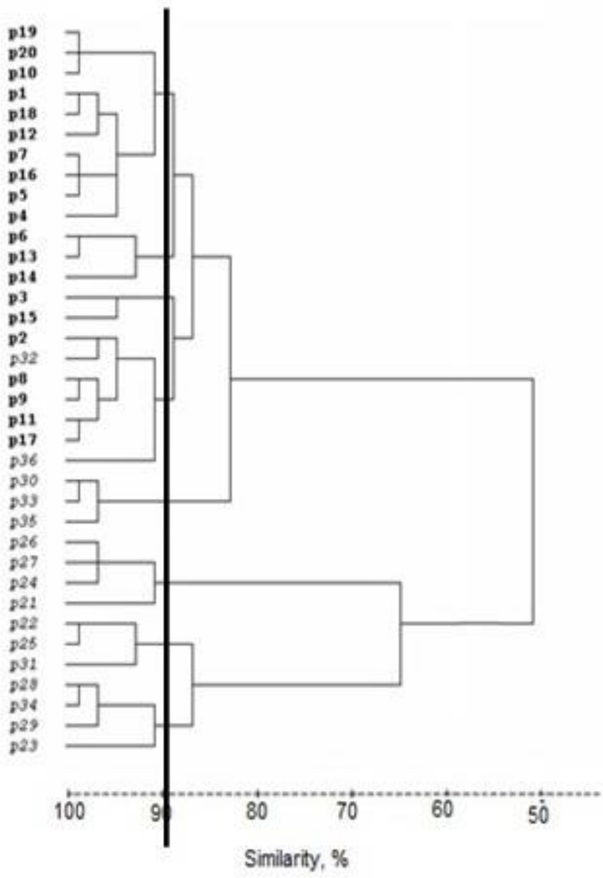

Figure 4. Phenotypic dendrogram derived from Euclidean distance and UPGMA method. Codes in bold indicate local populations and codes in italics indicate introduced ones.

Table 4. Pearson correlation coefficients between all studied characters

Characters	FM	DW	Stem length	Stem. plant^{-1}	LC	WC	FS	Leafs no	Emer-gence	Inf plant^{-1}
DW	-0.739**									
Stem length	0.221	-0.105								
Stem. plant^{-1}	0.397*	-0.419*	0.260							
LC	0.479**	-0.423*	0.367*	0.390*						
WC	-0.220	0.441**	-0.128	-0.091	-0.232					
FS	0.014	-0.014	0.011	0.282	0.351*	0.176				
Leafs no	0.606**	-0.701**	0.317	0.443**	0.633**	-0.391*	0.124			
Emergence	0.141	-0.420*	-0.053	0.138	0.078	-0.162	-0.081	0.499**		
Inf plant^{-1}	-0.452**	0.727**	0.029	-0.058	-0.331*	0.378*	0.055	-0.430**	-0.337*	
Pod plant^{-1}	-0.530**	0.755**	0.043	-0.175	-0.402*	0.288	-0.142	-0.540**	-0.372*	0.830**

**: Correlation is significant at the 0.01 level. *: Correlation is significant at the 0.05 level.

DISCUSSION

Alfalfa (*Medicago sativa*) is an autotetraploid, allogamous and heterozygous species whose cultivars are synthetic populations (McCoy & Bingham, 1988). The erosion of genetic diversity in most of our cultivated species underlines the need to describe genetic variation through collection material, which could be used in breeding programs. For this, a collection of 36 native and foreign alfalfa populations was investigated using agro-morphological traits. Results were subjected to variance analysis, and Principal Component Analysis (PCA) to investigate the relationship between the native and introduced populations.

Several studies reported a wide inter-population variation among alfalfa local populations and varieties (Julier and Huyghe, 1997; Julier et al., 2000; Annicchiarico 2006), and confirmed the importance of selection for exploiting the genetic resources of lucerne. By ANOVA and CV indices analysis, our results showed a high diversity in the agro-morphological traits among the 36 populations. Furthermore, a significant difference between local and exotic ecotypes was recorded. Compared with native populations, the introduced ones were characterised by high green matter yield, high stem number, low dry matter percentage and short stems. These results could explain the importance of using the varieties selected in North Africa, which are very productive and adapted to arid conditions (Elgazzah & Chalbi, 1995). The variation assessed among populations seems attributed to the differences in their genetic make-up and to environmental adaptation. The environmental adaptation of ecotypes can be related to three factors: (i) arid conditions (high temperature, summer drought), (ii) biotic interactions (weeds species, legume-*Rhizobium* interaction) and (iii) the cutting frequency. In Tunisia, the local ecotype generally undergoes frequent cuts which can exceed 7 cuts per year (Haddad et al., 2003). In contrast, in North Italy, the mowing frequency for a native landrace called 'Romagnola' ranges between 2.5 to 4 harvests per year (Annicchiarico, 2006).

In contrast, results related to reproductive components showed that exotic varieties had lower values than the native ones. The pod per plant for local populations was six times higher than for the introduced ones (Table 3). The advantage in inflorescences and pods per plant observed in local landraces is not in agreement with the low seed production in Tunisia (200 kg/ha) (Seklani et al., 1990). Therefore, the major problem of commercial introduced varieties is their no-regeneration for new generations.

Multivariate analysis has been used to group populations in genetic diversity studies (Garcia et al., 1997; Bayuelo-Jimenez et al., 2002; Mohammadi and Prasanna, 2003). Principal component analysis is often used before cluster analysis to determine the relative importance of variables classification (Berdahl et al., 1999; Bregard et al., 2001). In our study, results of PCA showed that dispersion of populations was organised in two types of genotypes pools, local and foreign ones, completely distinguished, except for P36 from Morocco. By clustering analysis, the populations were grouped into eight clusters. In the fourth cluster, the two foreign cultivars, Magali (P32) from France and Africaine (P36) from Morocco, were grouped with the native populations. This result can be explained by the fact that these populations were probably used in Tunisia since old times and were inter-crossed with native alfalfa. Consequently, this germplasm could be considered the source of genetic erosion of the local ecotype called '*Gabssia*', which has a long history of cultivation worldwide and it has shown wide range of adaptation to different environmental conditions (Bolaños et al., 2000). Populations from South Europe are mostly varieties bred for improved forage yield and adaptation (Julier et al., 2010). The other clusters with local populations consisted of accessions collected from the Tunisian sites. Therefore, the geographic origin did fit approximately with the obtained phenotypic dendrogram. Contradictory results were found by Crochemore (1998) who reported that the geographic origin of the vegetal material was sufficient to obtain a reasonable structure in groups.

The success of selection depends on the choice of selection criteria for improving seed yield (Samonte et al., 1998). For this reason, it is clear that a correlation coefficient, which measures the simple linear relationship between two traits, can contribute to the selection success. In our study, correlations were highly positive between fresh yields and leaf number (indicator of digestibility) on one hand, and between dray matter percentage and reproductive traits ion the other hand. This result indicate the possibility of combining high yield and good digestibility or high dry matter and seed yield in one genotype growing under arid environment.

From an applied perspective, our results constitute over-wide information for native alfalfa landraces cultivated in Mediterranean regions and this collection could be used in many breeding researches. Julier et al. (2000) confirmed that in alfalfa three possibilities can be considered (i) Individual evaluation of forage quality on spaced plant on nursery, (ii) cloning of individual plants by stem cuttings and evaluation of clones planted in a replicated experimental design, and (iii) progeny tests. As perspective to this work, a selection of new variety from this collection is necessary. For this, we have established a breeding program in IRA (Institute of Arid Regions, Tunisia), where alfalfa populations were installed under drought conditions (low frequency of irrigation) to choose the tolerant plants that survive and produce good yields. Those plants will be used to produce inbred lines. They will be crossed to develop new synthetic cultivar with "high yield and tolerant to drought and heat".

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