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DIVERSITY OF LUCERNE (*MEDICAGO SATIVA* L.) POPULATIONS IN SOUTH TUNISIA

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Abstract

Twenty cultivated populations of lucerne (*Medicago sativa* L) collected from different oasis of Tunisian south were evaluated for morphology and yield. Important among-accessions variation was observed for all traits by variance analysis (length and width of central leaflet at flowering, length and diameter of stem, growth habit, date of flowering, weight of 1000 seed and total fresh and dry matter) when population effect was highly significant ($p<0.05$) excepted four i.e., stems number, number of smell by inflorescence, number of inflorescences by cluster and plant colour. The large least significant difference at 5% values indicate that a large proportion of this variability can be attributed to genetic variability between individual plants within an accession. Principal component analysis (on three axes represented 59.85% of the total variation) and cluster analysis, based in significant traits, show the distribution of the populations is not according to their geographic origins. Correlation between yield and morphological trait shows that dry matter is negatively correlated to stem length (-0.61) and positively to stem diameter (0.43). Fresh matter was negatively correlated to seed weight (-0.31) and positively to leaf dimension. This work will be completed by a selection programme in these twenty accessions for the improvement of the alfalfa cultivated in the Tunisian south.

Introduction

Lucerne (*Medicago sativa* L.) is a very used forage legume with over 32 million hectares in the world (Michaud *et al*, 1988; Barnes *et al*, 1977). In Tunisia, it is the most cultivated forage legume, grown over 12410 hectars (Ministère de l'environnement et du developpement durable, 2007). It is cultivated primarily in the oases. It is highly salt tolerant (Janati, 1990; Mezni *et al*., 2002; Aganga & Tshwenyane, 2003) and is widely adapted to southern Tunisia farming regions (oases). There are large areas where growing lucerne would be beneficial but its use is constrained to environmental stresses, such as high water salinity and drought (Garnett *et al*., 2004). Lucerne has been cultivated in oases (arid region) of Tunisia since many years, and is currently grown on more than 9720 hectars across Southern Tunisia (Anon., 2005). The accessions genetic diversity of arid regions is considered as an important genetic source for drought and salinity resistance for future breeding (Skourie 1988; Leberre & Ramousse, 2003; Anon., 2004). Information on the genetic structure of populations for adaptive and yield traits have noticeable implications on the strategies for germplasm collection, conservation and exploitation.

Several factors determining the genetic diversity of plant species, the geographic range is one of the major of them (Karron, 1987; Hamrick & Godt, 1990). Many studies have revealed that narrowly restricted species tend to have a lower level of genetic diversity than the widespread congeners (Karron, 1987; Sherman-Broyles *et al*., 1992; Linhart & Premoli, 1993; Edwards and Wyatt, 1994; Godt *et al*., 1997). A few studies, however, have reported the opposite idea (Ranker, 1994; Lewis & Crawford, 1995).

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The genetic diversity in *Medicago sativa* have been estimated using morphological and yield traits (Julier *et al*., 2000; Bola˜nos-Aguilar *et al*., 2000), histological traits (Guines *et al*., 2003), RAPD markers (Crochemore *et al*., 1998) and allozyme markers (Jenczewski *et al*., 1999). These studies have shown that *Medicago sativa* is characterized by high within population variation. Instead of molecular markers, genetic variation in populations can be investigated by assessing quantitative variation that is under polygenic control where many loci and the environmental effects on those loci, contribute to the quantitative variation in the traits being investigated (David, 2005). The purpose of this study was to examine the morphological and yield variation of lucerne accessions collected in the South of Tunisia, in order to complete genetic resources conservation and the evaluation of native germplasm of lucerne (Permed, 2004) and constitute a base for breeding program.

Material and methods

a. Plant material: The prospection concerned the oases of south Tunisia in Gabes, Kebili and Tozeur (Fig. 1) aim to complete genetic resources conservation and to evaluate a native germplasm of lucerne (Anon., 2004). Twenty local accessions (Table 1) collected from the three provinces were stored. At the same time, semi-structured interviews were carried out with farmers who donated the samples at each collection site. These reports consisted of detailed information on local cropping practices, special characteristics of local lucerne and its use.

Accession means a sample or population of plants species collected and conserved in our "genes banc" of IRA (Institut des Régions Arides) to a future evaluation and improvement. Px is the identifying number of accession. "P": First letter of population. "x" : Random Number attributed to each accessions.

b. Experimental design: This study was carried out at experimental field of IRA (South of Tunisia). Each accession was sowed on four rows (4 x 2 m) in September 2005. The characterisation and mineral composition of water irrigation of experimental site are presented respectively in Tables 2 and 3. Field trial consisted of 650 m^2. Trial was carried on randomised complete block design with 4 replicates. Plots were sown by hand and sowing depth was 0.5–1 cm. Plants were surface-irrigated at regular intervals of 25 days (oasis rhythm). Trials were hand cleaned to get rid of weeds.

c. Morphological and yield traits: Measurements were carried out at a vegetative and reproductive stage during 2005 and 2006. Accessions were scored on different morpho-agronomic characteristics according to the descriptors for forage legumes, published for CEC and IBPGR (1984). These traits were:

- LF: Length of central leaflet at flowering
- WF: Width of central leaflet at flowering
- SF: Shape of leaf; 3(= elongated), 5(=ovate) and 7(=round)
- SN: Stem number
- SL: Stem length
- SD: Stem diameter
- PC: Plant color (1= Dark, 2=Clear and 3= Speckled)
- GH: Growth habit (GH) in autumn of the first year (1= erect, 3= semi erect, 5= medium, 7= semi prostrate and 9= prostrate)

- DF: Date of flowering in days after the beginning to June
- NS: Number of smell by inflorescence
- NI: Number of inflorescences by cluster
- WS: Weight of 1000 seed

Yield for each accession was evaluated by total dry matter (TDM) and total fresh matter (TFM), during six harvests in growing season 2005-2006.

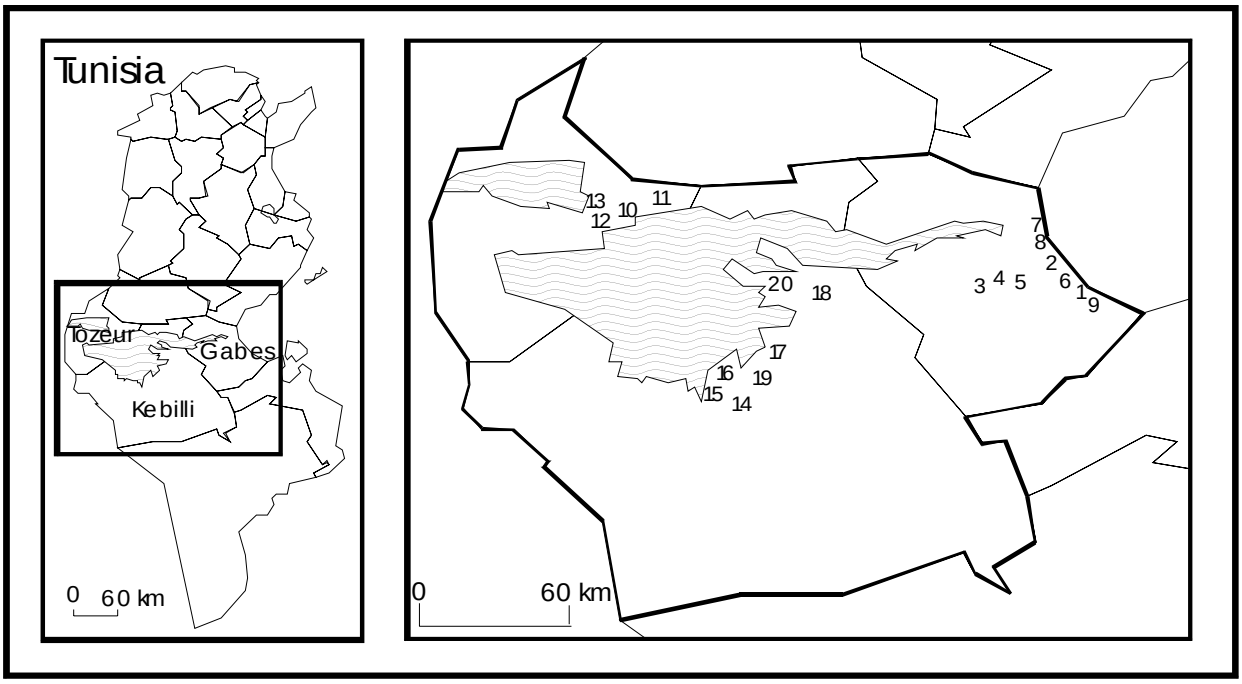

Fig. 1. Collecting sites of local lucerne, in the south of Tunisia. 9 accessions collected from Gabès, 7 collected from Tozeur and 4 from Kebili.

Table 1. Lucerne accessions collected from the oases of Southern Tunisia.

Codes accessions	Oases	Oases type	Province	Collection months in 2004	Seed quantity (kg)	Altitude m a.s.l.
P_1	Kattana	Coastal	Gabès	June	0.5	0 to 10
P_2	Chenchou	Coastal	Gabès	November	1	0 to 10
P_3	Chenini-1	Coastal	Gabès	February	0.5	0 to 10
P_4	Chenini-2	Coastal	Gabès	February	0.5	0 to 10
P_5	Chenini-3	Coastal	Gabès	February	0.5	0 to 10
P_6	Tboulbou	Coastal	Gabès	March	0.5	0 to 10
P_7	Metouia	Coastal	Gabès	April	0.5	10 to 20
P_8	Ghannouch	Coastal	Gabès	April	0.5	10 to 20
P_9	Zerkine	Coastal	Gabès	June	0.5	0 to 10
P_{10}	Essdada	Continental	Tozeur	December	0.4	−20 to −15
P_{11}	Bouhlel	Continental	Tozeur	December	0.4	−20 to −15
P_{12}	Dgach	Continental	Tozeur	December	0.5	−20 to −15
P_{13}	Hammajerid	Continental	Tozeur	December	0.5	−20 to −15
P_{14}	Zaafrane	Continental	Kébili	November	0.5	−20 to −15
P_{15}	Nouael	Continental	Kébili	December	0.5	−20 to −15
P_{16}	Jersine	Continental	Kébili	December	0.5	−20 to −15
P_{17}	Elgolaa	Continental	Kébili	November	0.5	−20 to −15
P_{18}	Limaguess	Continental	Kébili	April	0.5	−20 to −15
P_{19}	Douz	Continental	Kébili	March	0.5	−20 to −15
P_{20}	Stiftimia	Continental	Kébili	April	0.5	−20 to −15

Table 2. Trial site characteristics in El Nahal (IRA-Gabès).

Location	Site	Mean temp. min °C (winter)	Mean temp. max °C (summer)	Mean rainfall	Soil
Gabès	El Nahal	12	28	180 mm	Sandy

Table 3. Mineral composition of irrigation water.

CE à 25°C	pH	Ca	Mg	Na	K	SO_4	Cl	CO_3H
3.9 mS/cm	7.6	394	96	423	23	1190	568	167

d. Statistical analysis: The data were analysed by means of the SAS GLM procedure. We have used the model of variance with one characters of classification (Dagnelie, 1975) to demonstrate the variability caused by genotype. The total variance was indicated by mean squares (MS total) and composed between variance population (MS between) and within variance populations (MS within). The compute of F-Ratio (F_R), compared to critical value (Fc) at test wise α=0.05, was used to determinate if there was a population effect and to compare between and within variance.

The means accession values of each character was compared with least significant difference ($LSD_{0.05}$) test. The LSD was used to determine if the difference between two accessions was large enough to be considered real at a fixed level of confidence ($LSD_{0.05}$ = 95% confidence). The structure of genetic variation among the accession, was analysed using a principal component analysis (PCA), which was based on the means accession values across the four replications. The clustering of cultivars into similarity groups using method of UPGMA (Unweighted Pair-Group Method, Arithmetic Average) was established by XLSTAT which was obtained using average linkage and correlation coefficient of Pearson.

Results

a. Variance analysis and means comparison: The ANOVA for all experimental data in oases conditions at first year can estimate the variation between different lucerne populations of south Tunisia. The variance analysis (ANOVA), presented in Table 4, show that $F_R > 1$ for all characters. So, we must reject hypotheses H_0 (population effect).

For all characters, there were significant differences among *Medicago sativa* populations ($p<0.001$) except four characters, when the $F_R < F_c$ (5%). These characters are stems number (SN), number of smell by inflorescence (NS), number of inflorescences by cluster (NI) and plant colour (PC). So, there is among-population heterogeneity during the first seasonal growing for the majority of examined characters. The variance analysis was completed by averages comparisons for all characters with LSD test (0.05).

Table 5 presents the means values and the least significant difference (LSD) of significant traits, tested at different studied populations. The large $LSD_{0.05}$ values indicate that a large proportion of this variability can be attributed to genetic variability between individual plants within an accession. This test indicated that central leaflets at flowering of P10 were significantly elevated and of P19 were significantly withheld, compared to other populations. Concerning shape of leaf (SF), the percentage for each shape on all measurements was: 49 % ovate, 46 % round and 5 % elongated. The stem characters values vary from 7 (P19) to 8 (P5) for the number, from 49.4 (P15) to 83.6 (P5) cm for the length and from 0.12 (P10) to 0.4 (P8) cm for diameter. P1 and P18 populations had a maximum prostrate tendency of growth habit (GH) in autumn. For date of flowering per days after the beginning of June (DF), P11 and P20 are the earliest, P4 is the latest. WS is measurement on the collection sample, varies from 2.17g (P1) to 2.62g (P18).

Table 4. Analysis of variance (ANOVA); df, MS and Factor ratio.

Characters	Source of variance	Df	MS	F_R
LF	Between	19	0.365	11.39*
	Within	180	0.032	
WF	Between	19	0.086	7.48*
	Within	180	0.011	
SF	Between	19	5.695	10.64*
	Within	180	0.535	
SN	Between	19	16.19	1.45ns
	Within	60	11.18	
SL	Between	19	946.54	10.63*
	Within	180	89.077	
SD	Between	19	0.0217	7.34*
	Within	60	0.0029	
PC	Between	19	0.806	1.41ns
	Within	180	0.572	
GH	Between	19	6.32	3.31*
	Within	180	1.90	
DF	Between	19	8.749	3.48*
	Within	60	2.512	
NS	Between	19	24.571	1.27 ns
	Within	180	19.353	
NI	Between	19	6.409	1.72 ns
	Within	180	3.733	
WS	Between	19	0.060	infini*
	Within	60	0.000	
TFM	Between	19	1466249.711	8.057*
	Within	60	181983.377	
TDM	Between	19	139.579	2.632*
	Within	60	53.037	

$F_{critical}$= **2.00 (**df=19,60 and $\alpha = 0.05$) ; $F_{critical}$= **1.97** (df=19,180 and $\alpha = 0.05$)
*: Significant, ns: No-significant

The population effect is openly significant for production traits ($p<0.01$). An important variance intra-population from total variance for TDM was observed with 40 %, and it was weak for TFM with 12.5 %. Total seasonal fresh matter in all harvests was variable between 3.70 Kg/m^2 (P3) and 5.93 Kg/m^2 (P10). Total dry matter was variable between 165.45 $^0/_{00}$ (P7) and 189.35 $^0/_{00}$ (P20).

b. Principal component analysis: Grouping of lucerne genotypes using PCA was based mainly on the first three PC. Table 6 shows the contribution of different significant characteristics to each PC. These axes represented 59.85% of the total variation, respectively 24.75, 18.89 and 16.20%. The first component was positively correlated essentially to data of total fresh matter (TFM), length and width of central leaflet (LF and WF) and stem length (SL). Correlation between PC1 and shape of leaf (SF) was negative. PC1 separated accession having height TFM as P1, P10, P11 and P12 from those with low ones as P3, P6, P9, P14 and P15.

Table 5. Mean value and LSD (0.05) of significant morphological characteristics, total fresh matter and total dry matter tested at different studied populations.

	LF*	WF*	SF*	SL*	SD*	GH*	DF*	WS*	TFM*	TDM*
P1	1,66	0,58	3,8	57,9	0,3	**7,4**	22,7	2,17	4854.63	171.17
P2	1,36	0,56	4,2	72,6	0,225	7	23,25	2,42	5591.88	173.96
P3	1,35	0,61	5	59,6	0,2	6,4	22,25	2,41	*3708.75*	176.46
P4	1,37	*0,15*	3	74,8	0,375	5,6	**24,79**	2,52	4130.63	174.00
P5	1,7	0,49	3,6	**83,6**	0,35	5,4	24,25	2,41	4630.13	170.94
P6	1,41	0,41	4,4	55,7	0,275	5	23,75	2,29	4423.00	181.87
P7	1,64	0,45	5	82	0,375	5,2	23,75	2,2	4597.25	*165.45*
P8	1,8	0,55	4	61,6	**0,4**	6,8	22,5	2,43	4541.88	179.67
P9	1,67	0,35	5	52,8	0,2	5,4	24,75	2,49	4465.63	179.72
P10	**1,87**	0,61	3	66,8	0,125	6,8	21,75	2,23	**5931.38**	172.51
P11	1,75	0,45	4,2	72,6	*0,15*	5,2	20,5	2,45	5097.13	168.45
P12	1,42	0,43	3	67,9	0,25	5,4	21	2,21	5276.63	172.07
P13	1,25	0,52	3	66,4	0,275	6,6	22,75	2,24	4585.75	177.03
P14	1,35	0,32	5	55	0,2	6,2	23,75	2,5	4185.00	175.05
P15	*1,21*	0,45	5	*49,4*	0,225	6,4	24	2,36	3809.88	177.84
P16	1,43	0,59	3,8	75,1	0,25	4,8	22,25	2,37	4630.63	168.83
P17	1,5	0,61	4	60,1	0,3	5,8	25,75	2,49	4890.88	175.55
P18	1,37	0,49	4,8	67,5	0,275	**7,4**	21,5	2,62	4997.13	170.12
P19	1,4	**0,64**	3,8	68	0,225	6	24,25	2,34	5633.13	185.43
P20	1,63	0,52	5	53,7	0,25	6,4	20,5	2,31	5539.50	**189.35**
$LSD_{0.05}$	0.158	0.095	0.645	8.328	0.076	1.218	2.242	0.00	2.364	1.871

ns: Not significant; *; Significant at the 0.05. $LSD_{0.05}$: Least significant difference.

Table 6. PCA axes defined by characters contribution.

		PC1	PC2	PC3
Inertia %		24,753	18,897	16,201
Cumulative %		24,753	43,650	59,851
	TFM	**0,799**	0,254	0,162
	TDM	-0,232	**0,788**	0,307
	LF	0,580	0,002	-0,019
	WF	0,558	0,391	-0,155
Traits defining the	SL	0,422	**-0,768**	0,253
axes	SF	-0,531	0,290	-0,387
	GH	0,175	0,345	**-0,676**
	DF	-0,577	-0,164	0,398
	WS	-0,546	-0,242	-0,297
	SD	-0,118	0,415	**0,747**

The most important variables integrated by PC2 were Total dry matter (TDM), width of central leaflet (WF), growth habit (GH) and stem diameter (SD). It is correlated negatively with stem length (SL). It was possible to differentiate, according to PC2, accession with short stem and height TDM (P6, P19, P20 …) from others with long stem and weak TDM (P2, P4, P7, P16…)

PC3 integrated characters related with stem diameter (SD) and relatively Total dry matter (TDM). It is negatively correlated to growth habit (GH). PC3 differentiated accessions with SD and TDM as P4, P5, P6, P7, P12, P16 and P19 from others with thick diameter of stem. It was distinguished accession with high TDM.

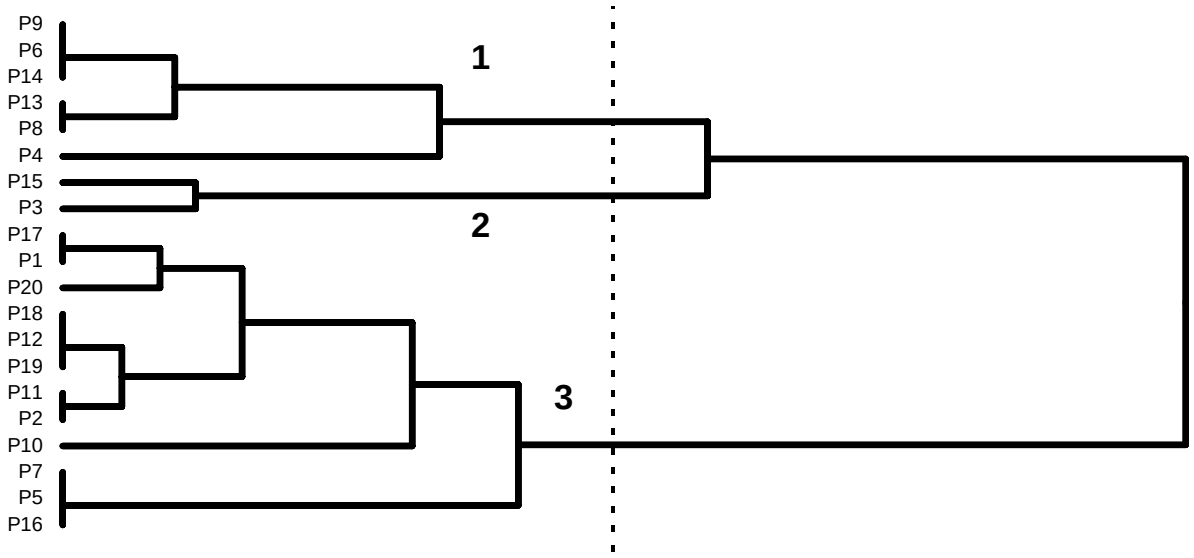

Fig. 2. Cluster dendrogram of South Tunisian accession

Table 7. Correlation between morphological and yield traits of 20 populations (correlations in bold are significant at 5%).

Variables	TFM	TDM	LF	WF	SL	SF	GH	DF	WS
TDM	0,090								
LF	**0,483**	-0,015							
WF	**0,411**	-0,009	0,178						
SL	0,186	**-0,611**	0,180	0,005					
SF	**-0,373**	0,147	-0,100	-0,047	-0,438				
GH	0,118	-0,039	-0,047	**0,345**	**-0,309**	0,035			
DF	**-0,419**	0,053	**-0,231**	**-0,241**	-0,030	0,039	-0,219		
WS	**-0,309**	-0,045	-0,169	**-0,316**	-0,037	**0,309**	0,030	0,242	
SD	0,146	**0,435**	-0,177	0,079	-0,077	-0,030	-0,248	0,272	-0,159

c. Cluster analysis: UPGMA produced a dendrogram with three main clusters (Fig. 2). The first cluster included accession P9, P6, P13, P14, P8 and P4. It shows many similarities in important TDM and SD. The second cluster grouped two accessions P3 from Gabès province and P15 from Kébili province characterized by weak production on TFM, short central leaf ant thin stem. The third cluster was composed of P1, P2, P5, P7, P10, P11, P12, P16, P17, P18, P19 and P20. All of them have high production of TFM, long stem and early flowering.

d. Correlation between yield and morphological traits: Table 7 shows correlation among the 10 variables. The most important correlation was negative, between stem length (SL) and total dry matter (TDM) with r=-0.61 at 5%, TDM was positive correlated to stem diameter. The total fresh matter had positive relation to LF (r = 0.48), WF (r = 0.41) and negative to SL (r = -0.37), DF (r = -0.42) and WS (r = - 0.31).

Other important correlations, which were weaker and significant at 5%, were seen between DF and LF, WF and SF, WF and DF, WF and WS, SL and GH, WS and SF.

These results indicate that it is possible to select promising materials for annual dry matter from obtained stem length. Also, it is possible to select promising materials for high annual fresh yield from the seed weight.

Discussion

The study of the morphological and yield characteristics of Tunisian Lucerne populations under oases conditions showed large heterogeneity among-population during the first seasonal growing for the majority of examined characters. Remarkable variation-within population was observed in some traits. Its contribution, of the total variance, was respectively 40.84% and 41.50% for stem number and plant colour, 44.06% and 36.44% for number of smell by inflorescence and number of inflorescences by cluster. Within variance was important for total dry matter (40%) but weak for fresh matter.

The wide detected intra-population variation reinforces the finding by several authors for the same or other traits. Julier *et al*., (2000) during six harvests with eleven cultivars, have reported a variance within cultivars from 57% to 100% for stem number and height. Crochemore *et al*., (1998) reported that the within population variation in alfalfa cultivars and landraces was larger than the among population variation. Estimates of within population and among-population variances have been reported by Julier *et al*., (2000) for forage yield and quality and by Bola˜nos-Aguilar *et al*., (2000) for seed yield traits of lucerne varieties, highlighting a trend towards larger variation within populations. The total variability that may be due, mainly, to interaction of several factors; (i) The reproductive aspect of the alfalfa (outcrossing) (ii) the uncontrolled introduction of the varieties improved in Tunisia (ElGhazzah et Chaibi, 1995); and (iii) the insufficiency of the seeds. The Lucerne culture is very old on the oases of the country. For a very long time, one cultivated only the local alfalfa of Gabes (Houérou, 1965). Later, several varieties were introduced and compared with the local population. Some of those made the subject of multiplication on the spot (case of "Provence", then "African" and "Moapa") who increased the risks genetic pollution of the populations of the south (Seklani, 1990).

Principal component and cluster analysis reveal a considerable variability. In each group, the accessions were different provinces. Genetic diversity observed in our analysis is not structured according to the geographic origins of population because the groups obtained in a classification aren't related to provinces. Cluster 2 obtained in dendrogram, for example, was formed by 2 populations; Chénini (P3) from Gabes province and Nouael (P15) from Kebili province (distant from 130 Km). Also, Zaafrane (P14) and Jerssine (P16) populations are very near (10 km) in some province, but was separated on 2 different clusters. This deduction is opposed to those findings in later studies, which have shown that the geographic origin of material was sufficient to obtain a reasonable structuration in groups (Barnes *et al*., 1977 and Julier *et al*., 1995). In other species like *Ceratonia siliqua*, Badragollo *et al*., (1997) confirm that genetic variation is not necessarily related to geographic variation and there was a wide interchange of plant material between different growing zones in the country.

Correlations between dry matter and fresh matter were positive and not significant at 5% (0.09) in the total harvests. Vodraska (1990), Rotili (1992), Johnson *et al*., (1994) and Julier & Huyghe (1997) found positive correlation between plant height and yield and negative correlation between plant height and quality. Our results showed that relationships between total dry matter and stem length are inversely proportional (- 0.61, 5%), and positive between fresh matter and stem length (0.18). Thus, the best production of dry matter could be evaluated starting from the shortest stems in oases conditions. It is possible to select promising materials for high annual fresh yield from the low weight seed (-0.31).

Conclusion

The information collected from the field evaluation and characterisation will make this collection as a considerable resource for future work (Trometter, 2000; Baudoin, 2001). To get height yield of agricultural crops under oases conditions (Drought and salinity stress), tolerant varieties are needed. So, there is sufficient variation in local Lucerne accessions for oases conditions to be increased substantially by selection and breeding. The genetic differences of individual plant (within-variance) found in our experiment indicated that variation could be used in breeding program for improved vegetative and reproductive traits. This results show that it is possible to developed improved cultivars by using suitable methods of hybridization. This work will be completed by a selection programme in these twenty accessions for the improvement of the alfalfa cultivated in the Tunisian south in IRA Gabes within PERMED project when genetic base was used to constitute parent selection and was included 60% from the best performing foreign populations in our trials of the total genotype.

For better variation characterization of Lucerne populations, this work will be completed by a molecular evaluation. In spite of these agronomic potentialities, the lucerne remains less studied in Tunisia. This evaluation is certainly important for better an agricultural valorisation of this leguminous plant in the Tunisia.

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