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Full Length Research Paper

Evaluation of forage biomass and seed yield among alfalfa progenies bred for adaptation to Tunisian outside oases conditions

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A breeding program focusing on the preservation and the improvement of local alfalfa germplasm '*Gabssia*' collected from different oases of southern Tunisia was undertaken since 2004. Exotic genotypes (*Sardi10, Ameristand801S, ABT805* and *Siciliano Ecotipo*) were used to improve local landraces considering their excellent response to the cropping conditions in the oases of southern Tunisia. In this trial, twelve superior alfalfa polycross progenies were evaluated on basis of polycross progeny performance. The purpose of this work was (i) to evaluate progenies' forage yield variability among different seasons of a single cropping year and (ii) to assess their seed yield components and shoots parameters during the last cutting. Obtained results showed a noticeable genetic diversity in almost of assessed traits. Significant differences on fresh and dry yields under progeny and season factors were recorded. Progeny has highly significant effect (P<0.05) on variation of all shoots traits expect fresh Leaf/stem ratio. For seed yield components, highly significant differences were obtained among progenies (P<0.01) expect 1000 seeds weights. Multivariate cluster analysis and ACP bi-plot showed four groups of progenies. Superior progenies belong to the first group (progenies of local genotype '*Gabssia'* L4, L23 and L39 having highest averages of seed yield components) and the second group (progenies of the genotype '*Ameristand801S'* A73, A17 and A56 characterized by the highest averages of forage yield and shoots parameters). Based on these progenies performances, the original clones are selected from superior plant material to establish a synthetic variety well adapted to the arid conditions outside oasis.

Keywords: alfalfa, forage, breeding, seed-yield, arid conditions.

INTRODUCTION

The perennial alfalfa (*Medicago sativa* L.) is regarded as the most cultivated fodder crop in the world such it can offer multiple agronomic and environmental assets with a wide adaptability (Annicchiarico *et al.* 2015).In the world, there are 30 Mh a fields in which alfalfa is cropped (Scasta *et al.* 2012). In Tunisia, it is cultivated on over 12410 ha (Benabderrahim *et al.* 2015, Abid *et al.* 2016).Genetic diversity of *Medicago sativa* was studied on the

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morphological, agronomic, histological and molecular levels (Moawed, 2016). Pre-studies showed that alfalfa is characterized by a wide variation within populations. Alfalfa is a leguminous plant, its fruits are pods. They are rolled up in whorls without spines contrary to the annual spontaneous species. They contain several yellow or brown seeds (Fourquin *et al.* 2013). Alfalfa's seed yield is considered among crucial criteria for forage seeds sellers. However, agronomic importance of this criterion is not yet well identified by producers. For breeders, it is of a secondary interest; such improving feed quality while maintaining high yield potential and good adaptability to biotic and abiotic stresses have been the major breeding program's targets (Annicchiarico *et al.* 2015). According to Annicchiarico *et al.* (2007) seed yields produced by alfalfa landraces are similar or superior than those of bred cultivars. Nevertheless, there is wide variability for seed yield among and within alfalfa cultivars (Bolanos-Aguilar *et al.* 2000).

Generally, Tunisian alfalfa producers harvest their own seeds from the last cutting of the cropping year. However, seed production and its quality are still uncontrolled and unreliable. Thus, a high level of genetic and physical purity and vital quality are needed to obtain a successful stand and high-quality certified seeds under all field conditions. The control of seed production can be explained by a number of individual yield components mainly the number of pods per plant, the number of seeds per pod, and seed weight. Hence, plant characters that increase pollination efficiency and seed yield have to be well understood and determined with a higher priority during breeding programs. Annicchiarico *et al.* (2015) indicated that selecting alfalfa for seed yield depends on seed yield per plant (highly related to seed yield per inflorescence) which displays narrow-sense heritability of around 0.50. Moreover, seed yield is correlated with number of seeds per raceme, raceme length and pod number (Boelt *et al.* 2015). On the other hand, studies of Bolanos-Aguilar *et al.* (2002) and Annicchiarico *et al.* (2013) indicated that genetic improvement for seed yield and forage yield (specifically biomass) at seed harvest stage are not antagonistic and displayed high (cultivar × environment) interaction.

Alfalfa has been cropped since a long time in oases of Tunisia. It occupies more than 9720 ha across the oases of the country (Tlahig *et al.* 2017). However, until today, the cropped alfalfa genetic materials have consisted only of farm landraces called '*Gabssia*'. But, according to Loumerem *et al.* (2015), these farm landraces are more effective for oases conditions than outside oases. The genetic structure of local landraces for adaptation and yield characteristics has significant implications for the strategy for collecting, conserving and exploiting this germplasm which can be used for breeding programs to establish highly drought and salinity resistant alfalfa varieties (Tlahig *et al.* 2017). Historically, local landraces '*Gabssia*' have been cultivated and showed an important adaptability to a wide range of environments around the world (Le Houérou, 1969).These landraces are a widely diversified genetic inheritance. Nevertheless, they have been threatened by the genetic erosion phenomenon. In this work, alfalfa local landraces and foreign varieties were evaluated and characterized on basis of poly-cross progeny performance. It was serving for a breeding program held in the Dry lands and Oasis Cropping Laboratory of the Arid Land Institute of Médenine, Tunisia. Twenty alfalfa '*Gabssia*' landraces were collected from different oases of southern Tunisia. Four exotic genotypes (*Sardi10, Ameristand801S, ABT805* and *Siciliano Ecotipo*) were introduced; considering their excellent response to the cropping conditions in the oases of southern Tunisia compared to the local landraces '*Gabssia*' and other North-African cultivars. Hundred plants originated from these germplasms were used as parental material in this breeding program to select synthetic varieties for arid regions of Tunisia based on polycross progeny performance (Loumerem *et al.* 2008). Seeds from polycross were grown in arid conditions outside oases to test their performance. Based on these progenies performance, clones from superior half-sib families were characterized to identify forage yield, feed quality, and salt tolerance. Favorable clones were used to achieve the breeding program regarding these selection criteria. In this trial, twelve superior progenies of polycross progeny test were evaluated on basis of poly cross progeny performance. The purpose of this work was (i) to evaluate progenies' forage yield variability among different seasons of one cropping year and (ii) to assess their seed yield components and shoots traits during the last cutting.

MATERIAL AND METHODS

Plant material and experimental design

The study was carried out in the experimental field of Dry land and Oases Cropping Laboratory in the Arid Land Institute of Médenine (33°29'57.80" N, 10°38'32.96" E, Altitude 16m). Based on the polycross progeny performance, seed of twelve alfalfa superior clones (Table 1) of one hundred local landraces and exotic varieties grown in performance tests were used in this trial (Tlahig *et al.* 2017).

The 12 progenies were 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 × 40 cm) on and between the rows. Irrigations were distributed twice weekly with equal quantities during the trial period. These progenies were evaluated at the same conditions measuring total fresh weight of the plant; dry weight of the stems and the leaves; number of stems; seed yield components.

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

Progenies	Half-sib family	Type	Geographic Origin	Selection criteria
L4 L23 L39	*Gabssia*	Landrace	Oasis, Tunisia	Adaptation to oasis conditions (Loumerem *et al.* 2015)
A17 A56 A73	*Ameristand 801S*	Variety	USA	Salt tolerance (Pecetti *et al.* 2013)
ABT21 ABT32 ABT52	*ABT805*	variety	USA	Grazingtolerance (Bouton *et al.* 2001)
E34	*Ecotiposiciliano*	landrace	Sicily, Italy	Adaptation to rain-fed conditions (Pecetti *et al.* 2008)
S47 S71	*Sardi10*	variety	Australia	High winter activity (Pembleton *et al.* 2010)

Seasonal forage yield comparison

To evaluate forage yield among seasons, alfalfa progenies were harvested 9 times during the cropping year (2012-2013). In summer and spring, cuttings were undertaken at one-tenth bloom stage; such the vigor would be transmitted to flowers to produce seeds. However, in winter and autumn, cuttings were scheduled for the time of development of new shoots from the crown. These cutting schedules allow good yields owing an acceptable feed quality with a minimal effect on stand persistence (Undersander *et al.* 2011; Jennings 2012).

During each cutting, fresh yields of all studied progenies were weighted using a precision balance (CelyweegschaalBB-P39/PL50, 30kg, d=1g). Then, from each progeny, a fresh pattern was weighted and then dried for 24 hours in an oven (Binder ED115) at a temperature of 105 °C.

Seed yield components

Seed yield components were evaluated on individual plant basis. At maturity, five plants per progeny on each block were randomly sampled. Five inflorescences of the plant were also randomly collected and numbers of pods per inflorescence were counted. Then, the first five pods of each inflorescence were sampled. These pods were threshed and their seeds counted to calculate the number of seeds per pod and the number of seeds per inflorescence. The rest of inflorescences from each sampled plant was threshed; and number of seed per plant was counted, the seed weight per plant, the 1000 seed weight and the mean seed weight were determined.

Shoot traits at maturity

Shoots were counted just after sampling and separating all inflorescences from each of the 15 plants per progeny (5 × 3blocks). Then leaves were taken apart stems to be fresh-weighted separately before being dried in oven at a temperature of 105°C until reaching constant dry weight.

Statistical analysis

Statistical analyses were carried out using SPSS 20.0 and Xlstat 2014 software. For the seasonal forage yields comparison, analysis of variance (ANOVA) was performed using GLM procedure. For the seed yield components and the pounded parameters of shoots, analysis of variances were computed using the one-way ANOVA tool. Analysis included only the progeny as fixed factor. Classification of progenies for each individual evaluated trait was realized by comparing means using Duncan multi-range test at (α=0.05).The structure of genetic variability among the progenies based on mean values of forage yield, shoots biomass and seed yield traits was analyzed using hierarchical cluster analysis (HCA) and principal component analysis (PCA) on the centered and standardized variants.

RESULTS

Seasonal forage yield comparison

Calculated values of $F_{0.05}$ show that there is a highly significant effect of season (*P*<0.001) on fresh and dry matter yields. Progeny factor reveals significant differences on fresh and dry yields (*P*<0.05).There is no significant difference due to the interaction (season × progeny) effect on both yield traits (Table 2).The purpose of comparing means is to know the performance of studied progenies within and between seasons. This test helps the breeder to know which progenies to select in accordance with criteria of productions. According to Duncan multi-range post-hoc test, there is no significant difference between spring and summer yielding whose scored the highest averages of production (Table 2). In fact, the difference among averages of fresh matter yield between spring and summer seasons was around 4%. Nevertheless, it decreased by 19.40% and 64.04% respectively at autumn and winter seasons compared with those of summer. Concerning the dry matter yield, the production of spring season was 9.17% higher than those of summer season. Whereas, it decreased by 11.17% and 66.41% respectively, during autumn and winter season. For all seasons, lowest averages were recorded for local '*Gabssia*' progeniesL23 and L4. Highest averages were observed for A17 and A73. Compared with the mean fresh yield of '*Ameristand801S*' progenies, averages of the progenies derived from '*ABT805', 'Ecotiposiciliano', 'Sardi10'*and local *'Gabssia'* were respectively 5.78%, 2.57%, 7.82% and 20.36% less than the mean of *Ameristand801S* fresh matter yield (1391.45 g/m^2). Eventually, for the dry matter yield, *Ameristand801S* progenies recorded the highest average (342.76 g/m^2) and it was 4.88%, 2.42%, 11.03% and 22.39% highest than the averages of other progenies derived from *'ABT805', 'Ecotiposiciliano', 'Sardi10'* and *'Gabssia'* respectively. The minimal fresh yield values was 194.67 g/m^2 and 209.6 g/m^2versus 49.35 g/m^2 and 48.74 g/m^2 of dry yield produced respectively by the progeny *ABT32* and S47 during winter period. Maximal values were 3808 g/m^2 of fresh matter yield and 1034.04 g/m^2 produced respectively by the progenies A56 and A17 of the genotype '*Ameristand 801S*' during summer period.

Shoots traits at maturity

Descriptive Statistics and ANOVA for shoot traits are presented in Table 3.

Progeny has a highly significant effect (*P*<0.05) on variation of all shoots traits (pounded parameters of leaves and stems and number of shoots per plant) expect fresh leaf/stem ratio. The leaves fresh weight varies from 4.00 g to 195.00 g/plant while stems fresh weight fluctuates between 3g and 219g/plant. On the other hand, the leaves dry weights were from 1g/plant to 87g/plant and from 1g/plant to 190g/plant for the stems dry weights. Highest leaves fresh weight (94.66 g/plant) and stems fresh weight (123 g/plant) were measured for A56 (Figure 1). The same progeny recorded the highest mean leaves dry weights (32.33g/plant) and stems (43.33g/plant).Concerning the number of shoots at maturity, averages fluctuated between 31.77 shoots for E34 and 93 shoots for A56. Progenies derived from *'Ameristand801S'* showed the highest numbers of shoots with an average of 83.72 shoots/plant followed by local *'Gabssia'* progenies whose averaged 50 shoots/plant. Expect ABT52, all progenies presented higher percentages of leaves than stems regarding their respective weights. In fact, according to these percentages, leaf/stem ratios fluctuated between 0.78 for A56 to 1.10 for ABT52. The progeny E34 derived from the genotype *'Ecotiposiciliano'* recorded the lowest averages of all shoot traits parameters.

The number of shoots per plant was highly correlated with leaves fresh weight (r=0.855, *P*<0.01), leaves dry weight (r=0.839, *P*<0.01), stems fresh weight (r=0.833, *P*<0.01) and stems dry weight (r=0.811, *P*<0.01) (Table 4).

Seed yield components

Analysis of variances for the different components of seed yield showed highly significant differences among progenies (*P*<0.01) expect 1000seeds weights. The progeny L4 of local '*Gabssia*' germplasm got the highest averages for all evaluated traits (Table 5).

Numbers of pods per inflorescence varied from 5.56 for A73 to 8.97 for L4. Pods contained mean numbers of seeds going from 3.63 to 5.24. Moreover, total number of seeds per plant averaged from 672 seeds for A73 to 2125 seeds for L4. Total seeds per plant were from 1.07g for A73 to 3.62 g for L4.However, 1000 seed weights did not exceed 1.74 g for all studied progenies. The progenies were arranged (Duncan, 5%) into four groups regarding seed weight/plant, as it is considered the most interesting trait (table 5).

Multivariate classification of studied alfalfa progenies

A multi-criteria classification seems to be necessary to succeed the breeding decision. For that, cluster analysis method used as multivariate statistical analysis has been suggested for comparisons of genotypes means using multiple parameters simultaneously (Ben Khaled *et al*. 2012). 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

Table 2. ANOVA and average comparison of seasonal forage yields among twelve alfalfa half-sib progenies bred in arid conditions (outside oases) of southern Tunisia during (2012-2013).

		Fresh yield (g/m^2)			Dry yield (g/m^2)		
		Mean	SD	Variance	Mean	SD	Variance
Season	1. spring	**1601.46 c**	546.27	298409.92	**421.29 c**	177.87	31638.42
	2. summer	**1668.33 c**	775.82	601899.25	**385.88 c**	152.88	23373.77
	3. autumn	1344.72 b	428.13	183298.01	342.74 b	123.1	15154.72
	4. winter	599.92 a	208.46	43454.21	129.58 a	42.97	1846.61
Progeny	L4	1153.20 ab	525.67	276327.1	272.20 ab	109.34	11955.19
	A17	1346.71 ab	747.77	559156.05	**345.26 b**	222.85	49660.12
	ABT21	1336.05 ab	625.44	391170.64	**340.63 b**	184.28	33960.63
	L23	1020.38 a	435.89	190001.92	249.74 a	105.47	11123.35
	ABT32	1325.88 ab	664.5	441561.98	326.67 ab	162.67	26461.07
	E34	1355.81 ab	714.85	511012.24	**334.45 b**	184.22	33938.24
	L39	1151.21 ab	640.33	410020.09	276.05 ab	131.87	17390.52
	S47	1250.74 ab	679.95	462331.92	290.64 ab	152.66	23306.02
	ABT52	1271.03 ab	683.11	466643.09	310.79 ab	170.43	29047.04
	A56	1282.26 ab	790.51	624901.56	312.00 ab	192.65	37114.61
	S71	1314.68 ab	674.95	455563.25	319.28 ab	171.5	29411.19
	A73	**1436.38 b**	669.16	447774.6	**340.26 b**	162.76	26492.29
ANOVA		Fresh yield			Dry yield		
Season		***			***		
Progeny		*			*		
season × progeny		NS			NS		

Significance: Non significant $^{NS}P>0.05$, significant at * $P<0.05$, ** $P<0.01$, *** $P<0.001$;
Letters following mean values indicate the groups given by the Duncan test at 5%.

Table 3. Descriptive statistics and ANOVA of shoot traits at maturity yields among twelve alfalfa half-sib progenies bred in outside oases arid conditions of southern Tunisia.

Parameter	**Minimum**	**Maximum**	**Mean**	**SD**	**Variance**	**F**	**Sig.**
Leaves fresh weight	4.00	195.00	43.03	35.43	1255.56	5.156	***
Leaves dry weight	1.00	87.00	17.40	13.29	176.67	3.602	***
Stems fresh weight	3.00	219.00	52.25	44.39	1970.12	4.445	***
Stems dry weight	1.00	190.00	21.39	21.05	442.93	2.120	*
Number of shoots	7.00	207.00	52.12	35.87	1286.86	4.926	***
Fresh Leaf/Stem Ratio	0.72	1.67	0.90	0.35	0.12	1.072	NS
Dry Leaf/Stem Ratio	0.51	2.00	0.98	0.49	0.24	3.293	**

Significance: Non significant $^{NS}P>0.05$, significant at * $P<0.05$, ** $P<0.01$, *** $P<0.001$;

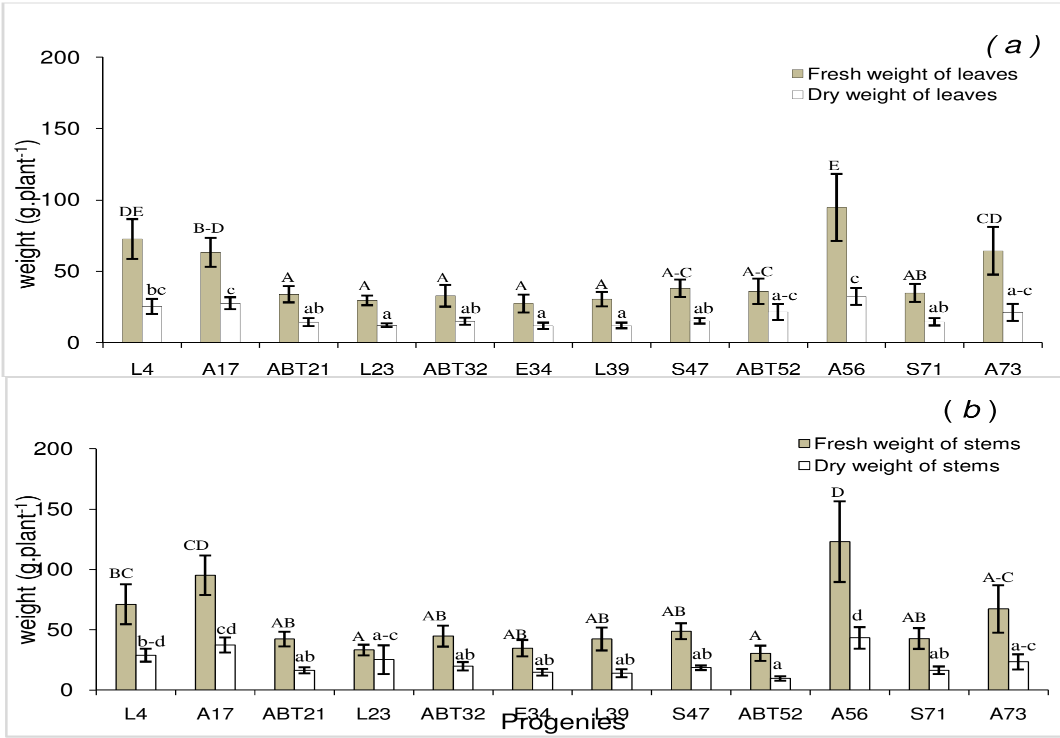

Figure 1. Leaves (*a*) and stems (*b*) fresh and dry weights of twelve alfalfa half-sib progenies bred in outside oases arid conditions of southern Tunisia. The histograms represent mean values (n=15), the error bars represent standard errors, the letters indicate the groups given by the Duncan test at 5%, in capital letters for the fresh weights and lowercase letters for the dry weights.

Table 4. Correlation between forage yield, shoots, and seed yield components traits

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Mean seasonal forage yield	1													
2. Leaves fresh weight	**0.869****	1												
3. Leaves dry weight	**0.847****	0.886**	1											
4. Stems fresh weight	**0.926****	0.860**	0.804**	1										
5. Stems dry weight	**0.910****	0.765**	0.826**	0.938**	1									
6. Fresh L/S ratio	-0.207*	0.022	-0.080	-0.326**	-0.365**	1								
7. Dry L/S ratio	-0.231**	-0.052	0.016	-0.282**	-0.361**	0.660**	1							
8. No. Shoots per plant	**0.877****	**0.855****	**0.839****	**0.833****	**0.811****	-0.108	-0.142	1						
9. No. seeds per pod	0.125	0.141	0.151	0.128	0.162	0.004	-0.064	0.171*	1					
10. No. pods / inflorescence	0.324**	0.316**	0.405**	0.262**	0.335**	0.024	-0.004	0.380**	0.562**	1				
11. No. seeds / inflorescence	0.306**	0.302**	0.369**	0.256**	0.332**	-0.011	-0.075	0.362**	**0.806****	**0.878****	1			
12. No seeds per plant	0.258**	0.291**	0.330**	0.261**	0.299**	-0.050	-0.050	0.314**	0.282**	0.424**	0.448**	1		
13. 1000 seeds weight	0.185*	0.129	0.232**	0.226**	0.319**	**-0.163***	**-0.189***	0.172*	0.164*	0.184*	0.194*	0.085	1	
14. Seeds weight per plant	0.286**	0.311**	0.368**	0.285**	0.347**	-0.065	-0.093	0.351**	0.284**	0.447**	0.465**	**0.979****	0.211**	1

Pearson's Correlation coefficients at 5%, followed by levels of significance:* P<0.05, ** P<0.01.

Table 5. Seed yield components of twelve alfalfa half-sib progenies evaluated in outside oases arid conditions of southern Tunisia. Values are means ± SE (n=15), the letters indicate the groups given by the Duncan test at 5%

Progenies	Number of seeds pod^{-1}	Number of pod sperinflere scence	Number of seeds per inflorescence	Number of seeds per plant (×10²)	1000 seeds weight(g)	seed weight per plant (g)
L4	**5.24 ± 0.14 e**	**8.97 ±0.29 d**	**47.63 ±2.22 f**	**21.25 ±4.84 c**	1.65 ±0.07 ab	**3.62 ±0.84 c**
A17	4.29 ±0.11 bc	7.51 ±0.34 c	33.00 ±1.79 c-e	14.24 ±4.24 ab	**1.74 ±0.07 b**	2.50 ±0.77 b
ABT21	4.79 ±0.16 c-e	7.31 ±0.17 c	35.39 ±1.53 de	14.83 ±3.64 bc	1.56 ±0.08 ab	2.18 ±0.50 ab
L23	4.96 ±0.16 de	6.93 ±0.17 bc	35.28 ±1.72 de	11.67 ±1.53 ab	**1.71 ±0.05 b**	2.03 ±0.30 ab
ABT32	4.27 ±0.19 bc	5.81 ±0.32 a	27.36 ±1.86 ab	11.79 ±2.03 ab	1.63 ±0.11 ab	2.00 ±0.37 ab
E34	4.59 ±0.19 b-d	6.09 ±0.19 a	28.52 ±1.51 a-c	7.49 ±2.26 ab	1.45 ±0.09 a	1.07 ±0.32 a
L39	3.63 ±0.22 a	6.23 ±0.42 a-c	25.67 ±1.99 a	11.19 ±2.09 ab	1.55 ±0.05 ab	1.80 ±0.34 ab
S47	4.89 ±0.19 de	7.12 ±0.24 c	36.16 ±2.12 e	13.97 ±2.26 ab	**1.74 ±0.06 b**	2.43 ±0.40 b
ABT52	4.87 ±0.15 de	6.29 ±0.24 a-c	31.49 ±1.72 b-e	8.61 ±1.33 ab	1.65 ±0.08 ab	1.41 ±0.21 ab
A56	4.60 ±0.21 b-d	6.28 ±0.29 a-c	30.52 ±1.84 a-d	9.26 ±1.74 ab	1.57 ±0.08 ab	1.52 ±0.30 ab
S71	4.25 ±0.18 bc	5.81 ±0.31 a	27.28 ±1.73 a-d	8.00 ±1.64 ab	**1.68 ±0.07 b**	1.38 ±0.33 ab
A73	4.09 ±0.25 ab	5.56 ±0.43 a	26.91 ±2.50 ab	6.72 ±2.55 a	1.57 ±0.08 ab	1.10 ±0.42 a
$F_{0.05}$	**6.557*****	**11.810*****	**10.398*****	**2.414****	**1.488^{NS}**	**2.493****

Significance : ^{NS} Non-Significant, significant at $^{**}P$<0.01, $^{***}P$<0.001

three principal components (PCs) gave Eigen values greater than 1.0 and explained 82.78% of the total variability among the progenies for all investigated traits. The first PC, which is the most important component, accounted for 46.46% of the total variability and was associated with forage yield and shoot traits at maturity. The second PC contributed with 24.37% of total variability explained by the variation in seed yield components.

The tow technique of classification used in this study (ACP bi-plot (figure 2) and multivariate cluster analysis on a level of dissimilarity d=18.5 (figure3) show four groups of progenies. The first group gathers progenies of the local genotypes '*Gabssia'* L4, L23 and L39. The second cluster is presented by progenies of the genotype '*Ameristand 801S'* A73, A17 and A56,The third group is formed only by the E34 progeny of the genotype '*Ecotiposiciliano*' and the last group is presented by the S47 progenies and S71 of the genotype '*Sardi10'* and progeniesABT21, ABT32 and ABT52 of genotype "*ABT805* ".

DISCUSSION

Introduced germplasm showed higher adaptations to wide range of Mediterranean environments. Their introduction to the current breeding scheme was to minimize inbreeding risk and the contribution to improve performances of native germplasm of oases in arid conditions (Tlahig *et al.* 2017).The results of this trials confirm the fact that local landraces "*Gabssia*" are more adapted to oases conditions (Loumerem *et al.* 2015) and their performances decreases when they misses the oasis' micro-climate (Benabderrahim *et al.* 2009, 2015). Benabderrahim *et al*. (2008) evaluated 16 alfalfa cultivars including *'Sardi10'*, *'Ameristand801S'*, *'ABT805'*, *'Ecotiposiciliano'* and local *'Gabssia'* for the adaptation to the oases conditions of southern Tunisia. Yields fresh matter of these genotypes under oases conditions were 2228.9 g.m^{-2}, 2116.7 g.m^{-2}, 1721.3 g.m^{-2}, 1366.8 g.m^{2} and 1230.4 g.m^{-2}produced respectively by *'Sardi10'*,*'Ameristand801S'*, *'ABT805'*, *'Ecotiposiciliano'* and local *'Gabssia'*. Averages of yields fresh matter produced by each half-sib progenies confirmed that there is a decrease of production under outside-oases compared with oasis conditions. Nevertheless, the percentage of this reduction varied from 0.80% for the genotype *'Ecotiposiciliano'*, which kept the same behavior under both environments, to 42.45% for *'Sardi10'* which recorded the highest production among studied cultivars under oases condition. Under outside-oases conditions, local *'Gabssia'* progenies produced 9.92% less compared with its production in oases. Despite the decrease of its production of 34.25%, *'Ameristand 801S'* remained the most adapted alfalfa variety for arid conditions of southern Tunisia. Annicchiarico (2015), Stavarache *et al.*(2015) and Pecetti *et al*. (2016) studied the affinity of leaves and stems percentages on the plant along growth stages in the field. In fact, leaves and stems keep constant percentages until the appearance of first blooming, then falling of leaves occurred from basal part of stems. That is why it is advised to harvest at pre-bud to one-tenth bloom stages to

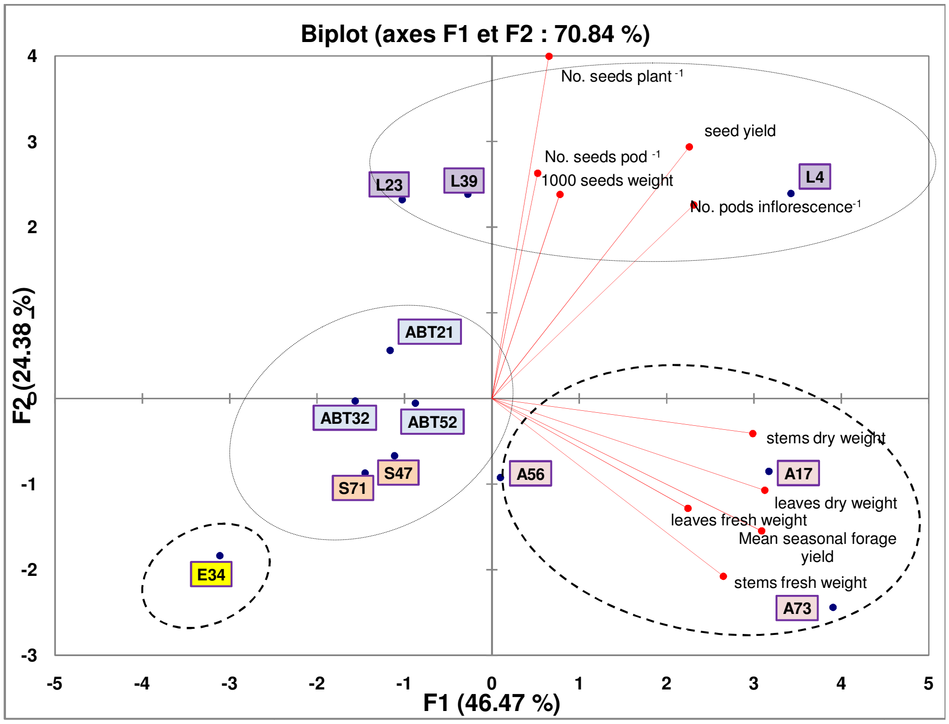

Figure 2. Principal component analysis (PCA) of forage and seed yield traits for 12 alfalfa half-sib progenies evaluated in arid outside oases conditions of southern Tunisia during2012-2013 cropping year.

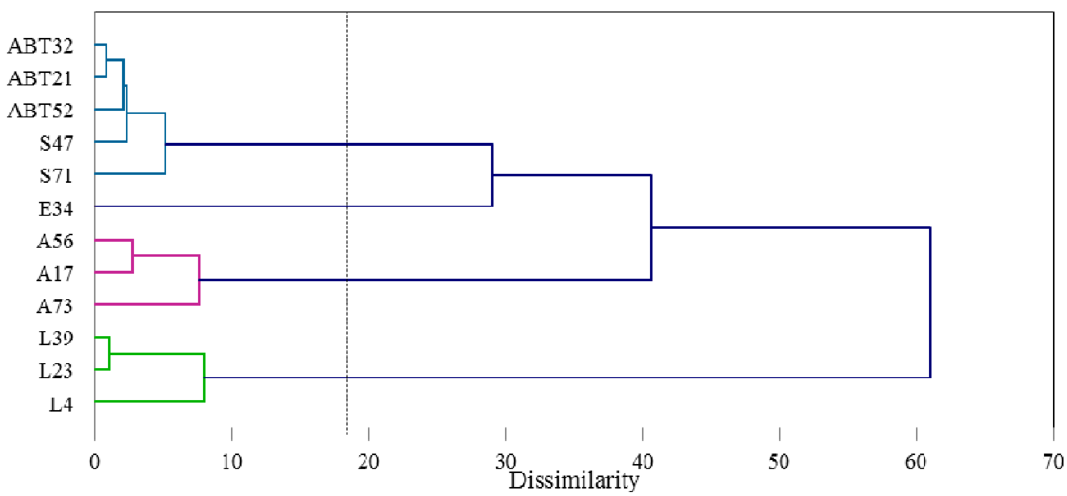

Figure 3. Hierarchical cluster (Multivariate cluster analysis) of 12 alfalfa half-sib progenies evaluated in outside oases arid conditions of southern Tunisia during 2012-2013 cropping year

guarantee interesting forage yields owing a good fodder quality (Loumerem *et al.* 2015). Hence, at full blooming stage, only inflorescences and few floors of leaves remain towards the top of the shoots. From the pre-bud stage until full flowering, the proportion of buds and flowers grows constantly. Moreover, if the plants are harvested at full flowering, the production will consist only of stems, inflorescences and only a few floors of leaves (Stavarache *et al.* 2015).

The different components of seed yield showed highly significant differences among progenies. Phenotypic variability for seed yield among alfalfa genotypes is due to crop management practices and high level of genetic impurity (Basafa and Taherian, 2009). Obtained results revealed that among-landrace variance of seed weight per plant (considered as the most representative seed yield trait) was about 29% while within landrace variance was around 46%. Bolanos-Aguilar *et al.* (2000) reported that among-population variance were accounted for 5 to 31% and within population variance for 69 to 95% of total genetic diversity in alfalfa for seed yield components. Moreover, there were significant correlations between most of pair wise seed yield components traits (Table 4). Highest coefficients of correlation were obtained between seed weight per plant and No. of seeds per plant (r=0.979, P<0.01), between No. of seeds per inflorescence and No. of pods per inflorescence (r=0.878, P<0.01) and between No. of seeds per inflorescence and No. of seeds per pod (r=0.806, P<0.01). Otherwise, Bodzon (2004) mentioned that the variability on the number of pods per raceme and seeds per pod determine about 60% of the seed yield variability among alfalfa genotypes. Other studies of Pelikán *et al.* (2014), Chocarro and Lloveras (2015), discussed the factors explaining seed yield variability in alfalfa.

Negative correlations were obtained between seed yield components and leaf/stem ratios. Nevertheless, coefficient of correlation was significant only for the couples leaf/stem ratio and 1000 seeds weight (r= -0.163 and r= -0.189, P<0.05) respectively with fresh and dry leaf/stem ratios). Similar results were obtained by Sheaffer *et al*. (2000) and Lamb *et al*. (2003) whose studied correlation between leaf/stem ratios and forage yields at different times of harvesting.

Multivariate cluster analysis and ACP bi-plot show four groups of progenies. The first group (the progenies of local genotypes '*Gabssia'* L4, L23 and L39) has the highest averages of seed yield and its components. Therefore, these progenies can be the starting genetic material for a breeding program attempting the improvement of seed yield and the selection of new alfalfa synthetic variety well adapted to the arid conditions outside oasis. The second group (the progenies of the genotype '*Ameristand801S'* A73, A17and A56) which are characterized mainly by the highest averages of forage yield as well as of pounded stems and leaves parameters used to determine leaf/stem ratios to predict forage quality, this results was reported by Annicchiarico *et al.* (2013), Annicchiarico (2015). The third group (the E34 offspring of the genotype '*Ecotiposiciliano*') is characterized by the lowest outputs of the majority of the studied characters. The last group (the S47 offspring and S71 of the genotype '*Sardi10'* and offspring ABT21, ABT32 and ABT52 of genotype "*ABT805* ") presenting high yielding during spring and summer period with an important winter dormancy.

CONCLUSION

In the current study, twelve alfalfa half-sib progenies, deriving from a breeding polycross set in Tunisian arid conditions outside oases, were assessed to compare their seasonal forage yield and their seed yield across 2012-2013 cropping year. Obtained results showed an interesting significant genetic variability between studied progenies for the majority of evaluated traits. Multi-criteria analysis permitted to classify alfalfa progenies regarding their characteristics. Under outside-oases conditions, local 'Gabssia' progenies produced 9.92% less compared with oases production. They were characterized by the highest seed yield components averages. Despite the decrease of its production of 34.25%, 'Ameristand801S' remained the most adapted alfalfa cultivar for arid conditions of southern Tunisia. It produced the highest forage yield averages and the best leaf/stem ratios. Consequently, superior progenies of 'Gabssia' and 'Ameristand801S' can be the starting genetic material for a breeding program attempting the improvement of biomass and seed yields and the selection of new alfalfa synthetic variety well adapted to the arid conditions outside oasis.

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