Original scientific paper
10.7251/AGSY1404324L

BREEDING OF LOCAL ALFALFA (*Medicago Sativa L.*) "GABSSIA" FOR YIELD AND IMPROVING TOLERANCE TO WATER SALINITY AND WINTER DORMANCY IN THE ARID REGIONS OF TUNISIA

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Abstract

Seed for growing a progeny performance test is obtained from one hundred clones that were selected from five genotypes of different geographic origins of alfalfa (*Medicago sativa* L.). Sixty percent selected from local cultivars "*Gabssia*" and forty percent from foreign varieties and landraces (*Sardi10, Ameristand, ABT805* and *Siciliano Ecotipo*). It was planted out in the Arid Institute of Research farm of Médenine. The objectives of this study were to assess the importance and effectiveness of progeny test used in alfalfa breeding for increased forages yield (fresh and dry matter). Results showed that the differences are highly significant (=0.05) for fresh and dry matter yields. The least significant difference ($LSD_{0.05}$) was also calculated to assess differences between progenies. Progenies were classified into two groups identified by cluster analysis.

Keywords: *Breeding, Genotype, Medicago sativa, Progenies.*

Introduction

In southern Tunisia alfalfa expansion is limited by environmental stress such as drought, water salinity and soil fertility (Mezni et al., 2002; Loumerem et al., 2007a).
A diverse range of local germplasm, from around the oasis of arid regions of Tunisia, has been collected and is being characterized and the seed multiplied in the period 2004-2007. Details of this collection, multiplication and storage were published (Loumerem et al., 2007a). Throughout those arid regions, small farmers grow local alfalfa (*Gabssia*) in areas with poor soils using traditional methods of cultivations. It is an important source of cash income to a large number of oasis farmers (Janati, 1990; Annicchiarico et al., 2011).
Sustainability of oasis farming in south Tunisia is under serious threat from the spread of soil salinity, declining soil fertility and problems with commercial introduced varieties of alfalfa. Recent studies have established local alfalfa (*Gabssia*) as the most suitable species to address those problems in the farming system of oasis (BenAbderrahim et al., 2009; Annicchiarico et al., 2011).
The aim of this project is to breed alfalfa cultivars specifically for those southern Tunisian oases. This region has an arid climate with cold and dry winter, and a long summer drought (M Timet and Escadafal, 1982). The breeding program is focusing on improving tolerance to water salinity and winter dormancy, as well as improving establishment with companion crops. Increasing yield remains an important goal in alfalfa breeding. The current method of alfalfa breeding is almost exclusively based on recurrent phenotypic selection, which involves intercrossing selected parents to produce a synthetic variety (Fotiadis, 1981 and 1988; Milic' et al., 2010; Milic' et al., 2011).

Synthetic varieties are widely used in alfalfa breeding. They are produced by growing together in an isolated plot usually 4-10 clones selected on the basis of the performance of progenies from a polycross.

Materials and Methods

This program is based on a collection of 20 accessions of local alfalfa "*Gabssia*" from oases of Tunisia. A detailed characterization of the accessions was given in the article (Loumerem et al., 2007a). For most studied variables, in particular yield, a significant difference was obtained between accessions.

Accessions were subject to a high saline environment (water of irrigation with high salinity) and plants survived and produced important economic field's yields are considered tolerant. Those plants were used to develop progenies. We included some germplasm derived from the best-performing foreign varieties and landraces in the arid oasis, considering the excellent response of some of those varieties in comparison with the local "*Gabssia*" and the other north-African cultivars. About 40% of foreign genotypes (*Sardi10, Ameristand, ABT805* and *Siciliano Ecotipo*) based on the variety responses over the second year at the site of evaluation (Annicchiarico et al., 2011). One hundred genotypes were used as parent in this experiment. Sixty best-looking plants from local "*Gabssia*" accessions and 40 plants of foreign varieties (Ten from each variety sited before) were selected for cuttings. alfalfa can be stem propagated without addition of hormones, as long as, the cuttings are taken from upper part of the stem, and they are maintained in humid environment (Combaud and Lelièvre, 2006; Loumerem et al., 2007b). Cloned plants were grown in spaced plantings and assessed in a polycross. The goal of progeny test is to assess parental components based on the value of the parents, hence their great importance in the breeding of perennial forage crops (De Araufo and Goulman 2002; Milic' et al., 2010b). Milic' et al., 2010 consider progeny test as the most suitable for breeding and developing synthetic varieties. Field trials were conducted at IRA's experimental field of El Fjé (Médenine). Seventy three progenies, for which sufficient seed was available, were used in the experiment. Each of the 73 progenies was sown on a long single row plot. They were arranged in randomized Complete Block Design with three replications. Each replication consists of 12 plants spaced 40 cm apart in a single row. Data of total fresh and dry matter yield were subjected to analysis of variance (ANOVA) of Agronomic parameters for all cuttings and for average yield for each season. The differences among the progenies were detected by LSD test. Dendrogram were used to classify progenies in similar groups by cluster analysis. During each cutting, fresh yields of all studied progenies were weighted using a precision balance. Then, from each progeny, we take a weighted fresh pattern, and then dried for 24 hours in an oven at a temperature of 105 °C (AOAC, 1973). There is no rule to follow when making the decision to cut; whereas, our decision to cut (in summer and spring) is taken just when the first flowering is seen such the vigor will be transmitted to flowers to produce seeds. But, under winter and autumn conditions (the weather is extremely cool), the growth of new shoots from the crown indicates that it is time to cut. Most authors indicate that alfalfa cut at one-tenth bloom is the best compromise between yield and quality without seriously reducing plant vigor and stand life. To retain high nutrition value of alfalfa, harvest at the proper growth stage is necessary. Harvest schedule in the range of one-tenth bloom in spring and summer to pre-bud stage in winter and autumn will result in acceptable yields of high quality feed with a minimal effect on stand persistence (Bosworth et al., 1992; Platt, 2005; Thiébeau et al., 2003; Orloff and Putnam, 2010; Undersander et al., 2011; Jennings, 2012).

Results and discussion

The aim of forage breeding programs is to maximize economic yield. “Therefore, harvest management of perennial alfalfa requires a compromise between quality and persistence. The intensity at which these forages are harvested, should depend on the nutrient needs of the livestock that will be consuming the forage, as well as, the life expectancy of the stand” (Bosworth et al., 1992).

The period between two consecutive cuttings vary from a minimum of 20 days in summer between the 8th and the 9th cuttings; and it reaches a maximum of 57 days in winter between the two last cuttings. The CV of the number of days between cuttings is 36.76% of the mean.

Calculated values of $F_{0.05}$ show that there is a highly significant difference between the studied progenies for the agronomic characters (table 1).

Calculated values of $F_{0.05}$ (Table 2) show that for a total yield of fresh matter and yield of dry matter (for all catting), inter-progenies differences are highly significant, therefore the progenies studied here are considered to be statistically different. But, for percent of dry matter, inter-progenies differences are not significant.

Table 1: ANOVA of Agronomic parameters for all cuttings

Characters	Sum of Squares	Degrees of freedom	Mean Square	F	Sig
Yield of fresh matter	89117705,08	72	1237745,90	5,20	,000
Yield of dry matter	3849900,87	72	53470,84	5,70	,000
Percent of dry matter	1579,73	72	21,94	1,27	,058

The least significant difference (LSD) is used to determine if the difference between two progenies is large enough to be considered real at a fixed level of confidence ($LSD_{0.05}$=95% confidence).

Use the appropriate $LSD_{0.05}$ value at the bottom of the (table 2) to determine true differences. Where the difference between two progenies within a column is equal to or greater than the $LSD_{0.05}$ value, it means there is a real difference between the two progenies averages.

The large $LSD_{0.05}$ values indicate that a large proportion of this variability can be attributed to genetic variability between individual plants within a progeny.

Progenies 45, 43, 40, 61, 47, 52, 66, 49, 21, 71, 60, 59, 64, 65, 42, 67, 25, 26, 10 and 16 were significantly better than all other progenies for yield of fresh matter and yield of dry matter. They represent all studied genotypes. The local progeny of the oasis cultivar “*Gabssia*” had significantly higher fresh and dry matter yields than foreign genotypes (*Sardi10*, *Ameristand*, *ABT805* and *Siciliano Ecotipo*). Six out of twenty progenies which have the higher forage yield (more than 40000g per year/8 plants) are local genotypes (table 3) followed by *Sardi10* (four progenies), *ABT805* (four progenies), *Ameristand* (three progenies) and *Siciliano Ecotipo* (three progenies). Nevertheless, the highest forge yield was given by *Ameristand* progeny 45 (55488g fresh matter and 11530 g dray matter per year/8 plants) and the lowest was given by local progeny 41 (27861g fresh matter and 6348 g dray matter per year/8 plants).

Calculated values of $F_{0.05}$ have demonstrated highly significant differences concerning both of yields of fresh matter and yield of dry matter produced by studied progenies at different seasons.

Higher yield progenies in the spring season are 45, 61, 60, 49, 71, 63, 43, 59, 64 and 65. Its scored more than 20000g per 8 plants and belongs to the following genotypes, two local “*Gabssia*”, two “*ABT805*”, two “*Sardi10*” and two “*Ameristand*”. In winter higher yield progenies are 47, 45, 40, 71, 30, 61, 38, 19, 48, 26, 21 and 52. They are not dormant and its yield more than 5000g per 8 plants. The highest yields (5952 g, 5825 g, 5598 g and 5446 g) were found in the progenies 47, 45, 40 and 71, while the lowest yields (less than 3000g) were recorded in the progenies 41, 15, 36 and 72. The highest yield progenies are foreign genotypes *Sardi10*, *Ameristand* and *Siciliano Ecotipo*, while the lowest yield progenies are local genotypes. This local germplasm were collected from oases. It has shown wide adaptation to arid oasis environments in Tunisia. They scored lowest yields in winter in the experimental field of IRA with different edaphic and climatic conditions than oasis "oasis effect" (Potchter et al., 2012). So we can conclude in this case they are less adapted to arid environment outside the oasis, in winter, than foreign genotypes (table 3).

Table 2: Yield of fresh and dry matter (Annual, Average, Minimum and Maximum yield) of studied progenies

		Yield of fresh matter (g)				Yield of dry matter (g)			
Genotypes	Progenies	Mean	Annual yield	Min	Max	Mean	Annual yield	Min	Max
L	1	898,38	35037	160,00	2429,00	199,07	7764	38,00	415,00
L	2	892,43	34805	390,00	2104,00	197,92	7719	105,00	398,00
S	3	853,82	33299	401,00	1941,00	198,94	7759	77,00	477,00
L	4	887,71	34621	244,00	1772,00	197,43	7700	67,00	367,00
L	5	954,64	37231	287,00	2309,00	196,02	7645	61,00	468,00
ABT	6	935,17	36472	300,00	2816,00	197,92	7719	70,00	498,00
L	7	952,43	37145	203,00	2148,00	204,89	7991	65,00	399,00
E	8	931,20	36317	360,00	2315,00	208,20	8120	82,00	403,00
S	9	841,92	32835	179,00	1905,00	189,10	7375	41,00	375,00
L	10	1032,25	**40258**	262,00	3021,00	223,82	**8729**	56,00	543,00
A	11	776,10	30268	233,00	1864,00	172,46	6726	49,00	377,00
L	12	907,94	35410	354,00	2537,00	189,02	7372	86,00	498,00
L	13	847,53	33054	89,00	2193,00	188,64	7357	16,00	449,00
E	14	940,69	36687	375,00	2443,00	201,94	7876	97,00	395,00
L	15	764,43	29813	244,00	2109,00	168,10	6556	53,00	359,00
L	16	1028,41	**40108**	276,00	2541,00	228,61	**8916**	55,00	468,00
A	17	976,66	38090	388,00	2313,00	220,23	8589	99,00	499,00
L	18	964,89	37631	384,00	2064,00	213,84	8340	85,00	421,00
L	19	899,23	35070	316,00	1730,00	200,58	7823	108,00	378,00
L	20	942,79	36769	258,00	2071,00	209,71	8179	56,00	343,00
ABT	21	1119,92	**43677**	262,00	2740,00	252,20	**9836**	60,00	697,00
L	22	826,58	32237	366,00	1601,00	183,89	7172	80,00	443,00
L	23	893,07	34830	442,00	2165,00	190,28	7421	98,00	405,00
S	24	766,07	29877	229,00	1782,00	178,92	6978	49,00	349,00
L	25	1040,23	**40569**	338,00	3578,00	227,38	**8868**	72,00	584,00
A	26	1036,97	**40442**	510,00	2036,00	225,82	**8807**	108,00	402,00
L	27	1013,89	39542	92,00	3270,00	217,48	8482	21,00	615,00
L	28	750,61	29274	262,00	1667,00	164,58	6419	79,00	359,00
ABT	29	957,33	37336	290,00	2112,00	211,41	8245	71,00	411,00
L	30	974,48	38005	309,00	2147,00	210,74	8219	86,00	401,00
L	31	916,89	35759	242,00	2750,00	203,12	7922	52,00	495,00
ABT	32	786,07	30657	233,00	1660,00	177,33	6916	51,00	358,00
L	33	1019,48	39760	533,00	2519,00	218,20	8510	121,00	390,00
E	34	1008,41	39328	410,00	2231,00	221,76	8649	83,00	497,00
L	35	767,66	29939	143,00	1911,00	171,12	6674	34,00	384,00
L	36	758,48	29581	260,00	1482,00	176,02	6865	81,00	323,00
A	37	869,41	33907	89,00	2281,00	191,43	7466	22,00	416,00
L	38	955,05	37247	431,00	2026,00	215,30	8397	96,00	449,00
L	39	897,87	35017	198,00	2122,00	199,20	7769	65,00	412,00
E	40	1183,97	**46175**	316,00	4298,00	263,71	**10285**	84,00	724,00
L	41	714,38	27861	113,00	2130,00	162,76	6348	22,00	380,00
E	42	1053,12	**41072**	276,00	2918,00	229,71	**8959**	64,00	515,00
S	43	1203,79	**46948**	192,00	4224,00	275,48	**10744**	46,00	922,00
S	44	891,12	34754	332,00	2032,00	194,58	7589	101,00	377,00
A	45	1422,76	**55488**	353,00	4108,00	295,64	**11530**	71,00	650,00
L	46	972,05	37910	381,00	2630,00	218,17	8509	88,00	574,00
S	47	1162,58	**45341**	593,00	2260,00	258,79	**10093**	113,00	535,00
L	48	980,64	38245	421,00	2590,00	211,38	8244	105,00	423,00
A	49	1126,10	**43918**	339,00	2639,00	240,07	**9363**	71,00	474,00
L	50	950,66	37076	279,00	2525,00	215,28	8396	90,00	436,00
L	51	1002,53	39099	284,00	2256,00	222,64	8683	87,00	418,00
ABT	52	1158,61	**45186**	486,00	2540,00	246,15	**9600**	104,00	420,00
L	53	1018,84	39735	276,00	2649,00	231,89	9044	56,00	475,00
S	54	909,17	35458	220,00	2685,00	203,84	7950	52,00	535,00
L	55	1001,20	39047	204,00	2370,00	219,51	8561	48,00	437,00
A	56	951,25	37099	256,00	2086,00	208,71	8140	92,00	405,00
L	57	1009,17	39358	265,00	3273,00	220,66	8606	62,00	590,00
L	58	978,00	38142	370,00	2070,00	213,76	8337	78,00	431,00
ABT	59	1089,17	**42478**	316,00	2817,00	241,46	**9417**	77,00	471,00
E	60	1089,43	**42488**	113,00	3692,00	235,15	**9171**	26,00	560,00
L	61	1175,41	**45841**	452,00	2672,00	255,89	**9980**	105,00	482,00

ABT	62	772,48	30127	252,00	2129,00	174,00	6786	58,00	317,00
E	63	1002,74	39107	318,00	3028,00	226,17	8821	72,00	600,00
L	64	1087,41	**42409**	243,00	2903,00	237,64	**9268**	84,00	501,00
ABT	65	1054,07	**41109**	202,00	2926,00	233,79	**9118**	46,00	585,00
S	66	1149,05	**44813**	266,00	3176,00	254,79	**9937**	56,00	676,00
L	67	1044,97	**40754**	341,00	2147,00	225,48	**8794**	64,00	423,00
L	68	1013,07	39510	451,00	2893,00	243,51	9497	100,00	647,00
E	69	933,30	36399	177,00	1883,00	210,02	8191	41,00	381,00
ABT	70	748,58	29195	225,00	1881,00	181,23	7068	63,00	519,00
S	71	1116,17	**43531**	273,00	3355,00	241,15	**9405**	56,00	591,00
L	72	809,76	31581	166,00	2046,00	181,89	7094	41,00	444,00
A	73	994,43	38783	248,00	2332,00	224,20	8744	59,00	472,00
LSD		218.35				43.47			

Table 3: Season yield of fresh and dry matter (average, minimum and maximum yield)

	Season yield of fresh matter (g)			Season yield of dry matter (g)		
Seasons	Average	Min	Max	Average	Min	Max
Winter	716,07	89,00	2113,00	163,55	16,00	476,00
Spring	1365,85	203,00	4298,00	295,13	65,00	724,00
Summer	884,80	151,00	3176,00	203,38	39,00	697,00
Autumn	698,21	92,00	4224,00	148,87	21,00	922,00
LSD	218.35			43.47		

The progenies 41, 15, 36 and 72 are considered dormant because during all winter season its scored between 2439 and 2995 g per 8 plants.

In summer, foreign genotypes (45, 21, 66, 40, 47 and 52) scored the highest yields. During autumn, progeny 43 (*Sardi10*) scored the highest yield (10211g) flowed by progenies 52, 40, 45, 26, 21 and 33, while the lowest yield is scored by *Ameristand* genotype (progeny 11).

Analyses of variance (ANOVA) were performed for two characters, the average season yield of fresh matter and average season yield of dry matter, between seasons showed high significant differences at α= 0.05 (table 4).

Table 4: ANOVA of yield of fresh matter (YFM) and yield of dry matter (YDM) for different seasons

		Sum of Squares	Degrees of freedom	Mean Square	F	Sig.
Average season yield of fresh matter (g)	Between Groups	220284228.47	3	73428076.15	394.725	.000
	Within Groups	528853475.55	2843	186026.745		
	Total	749137704.02	2846			
Average season yield of dry matter (g)	Between Groups	9762676.620	3	3254225.540	460.920	.000
	Within Groups	20070599.51	2843	7060.287		
	Total	29835189.91	2846			

Concerning yields of fresh matter, the highest average was in the spring (1365.85g), while the lowest average yield recorded in autumn (698.21g).

According to Bosworth et al. (1914), during the late summer alfalfa plants are preparing for winter by developing cold resistance and storing energy reserves in their roots.

Our aim of the above analysis is to know the behavior of studied progenies during different periods. Such evaluation informs the breeder which progenies may select in accordance with criteria of productions. For that, a hierarchical multi-criteria classification seems to be necessary to succeed the breeding decision. The previous dendrogram (Fig. 1) shows that progenies can be divided into two groups. The most homogenous progenies marked on the seasonal classifications belong almost completely to the second group of the global dendrogram. According to this classification enforced by a direct observation of vegetal material in the experimental site, we may select the superior progenies that can serve as plant material to achieve the breeding program.

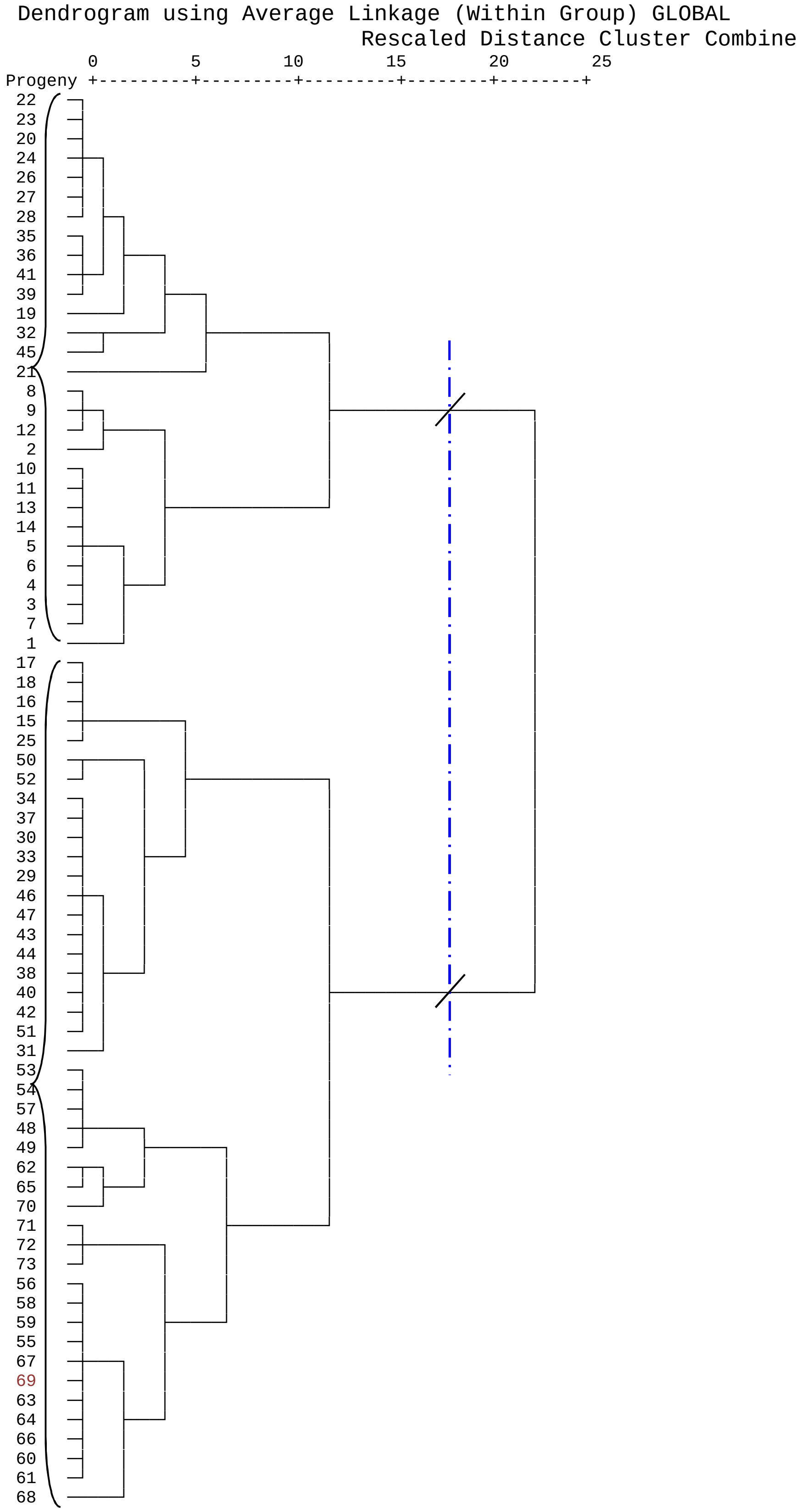

Figure 1 : Hierarchical classification of progenies for YFM, YDM and PDM

Conclusion

In spite of the agronomic and economic importance of the alfalfa, we do not have until today selected varieties adapted to the arid conditions except the oases landraces “*Gabssia*” which grows badly outside oasis. The present study consists of an agronomic and morphological evaluation of 73 progenies of alfalfa selected in IRA whose objective is to select best progenies with which the breeding scheme will be achieved. The analysis of the variances in terms of the characters of yields of fresh and dry matter show a highly significant difference between the studied progenies. An important genetic variability was noted after comparison the behavior of these progenies depending on the cutting seasons.

For all progenies, the spring yields were the most important. Summer productions are less important than spring yields for all progenies; it is in this season that alfalfa plants begin preparing for winter by developing cold resistance and storing energy reserves in their roots.

Hierarchical classification based on the criteria of yields in addition to the percentage of dry matter lets us distinguish two groups. The best group gathers the most homogenous progenies.

The 39 progenies that seem to be the best ones were selected.

For a further work, the selected progenies have to be propagated by stem cutting and transplanted in order to make the second polycross serving for the achievement of the breeding scheme.

Acknowledgements

This study was funded by the EU-funded project PERMED (INCO-CT-2004-509140). We wish to thank the farmers in the oases of Tunisia.

References

Annicchiarico P, Pecetti L, Abdelguerfi A, Bouizgaren A, Carroni A.M, Hayek T, M’Hammadi Bouzina M, Mezni M (2011). Adaptation of landrace and variety germplasm and selection strategies for Lucerne in the Mediterranean basin. Field Crops Research 120 (2011): 283-291

Association of Official Analytical Chemists “AOAC” (1973). Changes in Official Methods of Analysis Made at the Eighty-sixth. Annual Meeting, 3rd supplement to 11th Edition. Official Methods of Analysis-AOAC. Journal of the AOAC, Vol. 56, N° 2, 1973: 463-507.

BenAbderrahim M.A, Haddad M, Ferchichi A (2009). Diversity of Lucerne (Medicago sativa L.) populations in south Tunisia. Pak. J. Bot.. 41(6): 2851-2861

Bosworth S.C, Stringer W.C, Hall M.H, Fales S.L, Jung J.A (1992). Cutting management of alfalfa, red clover, and birdsfoot trefoil . Pennsylvania State Uni. Coop. Ext., 5Mrv49. Agronomy Facts 7. pp 20

Combaud S B & F. Lelièvre (2006). PERMED-Workpackage2. Note technique sur le bouturage de la luzerne. INRA France. Janvier 2006. pp 5

De Araújo M.R.A & Goulman B.E (2002). Genetic variation, heritability and progeny testing in meadow bromegrass. Plant breeding 121 (2002): 417-424

Fotiadis N.A (1981). Single plant versus clonal line performance in Alfalfa. Euphytica 30 (1981): 765-769

Fotiadis N.A (1988). Competition among components of synthetic varieties in alfalfa. Euphytica 37 (1988): 167-171

Janati, A., 1990. Les cultures fourragères dans les oasis (Forage crops in oasis). In Dollé V. (ed.), Toutain G. (ed.). Les systèmes agricoles oasiens. Montpellier: CIHEAM-IAMM, 1990: réf., tabl. (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n°

11). Séminaire sur les Systèmes Agricoles Oasiens, 1988/11/19-21, Tozeur (Tunisia). pp 164-169

Jennings, J., 2012. Alfalfa for Dairy Cattle. University of Arkansas Division of Agriculture. University of Arkansas Cooperative Extension Service Printing Services. Little Rock. Accessed August 5, 2012. To purchase print or efile, go to:
http://www.uaex.edu/Other_Areas/publications/PDF/FSA-4000.pdf

Loumerem M, Ferchichi A, Hadad M, Abdelrahim M.A, Hajjaji H (2007a) Collection and evaluation of Lucerne (Medicago sativa L.) germplasm from oases of Tunisia. Genet Resour Crop Evol (2007) 54: 1645-1651

Loumerem M, Tavares de Sousa M.M, Annicchiarico P, Hayek T, Boubakri C (2007b). Improvement of perennial forage plants for sustainability of Mediterranean farming systems. Lucerne (Medicago sativa L.) breeding work in south Tunisia. Options Méditerranéennes. Série A. N° 79 : 453-458.

Mezni M, Albouchi A, Bizid E & Hamza M (2002). Effet de la salinité des eaux d’irrigation sur la nutrition minérale chez trois variétés de luzerne pérennes (Medicago sativa) (Effect ot sodium chloride in irrigation water on the survival and on the weight and dimensional growth of three Lucerne cultivars. Agronomie 22 : 283-291.

Miliċ D, Katiċ S, Boċanski J, Karagiċ D, Valsiljeviċ S (2010). Importance of progeny testing in alfalfa breeding (Medicago sativa L.). Genetika, Vol 42, N°. 3: 485-492

Miliċ D, Mihailoviċ V, Karagiċ D, Valsiljeviċ S, Mikiċ A, Katiċ S (2011). Efficacy of progeny tests in alfalfa (Medicago sativa L.) breeding for yield and quality. Ratar. Povrt./ Field Veg. Crop Res. 48 (2011): 327-332

M Timet A & Escadafal R (1982). Carte des ressources en sols de la tunisie (feuille de Medenine). (Map of soils diversity of Tunisia (map of Medenine). Ministère de l’Agriculture. Direction des Ressources en eau et en sol. Division des sols. E-s 197. pp 26

Orloff S.B and Putnam D.H (2006). Harvest strategies for alfalfa. Inc.G. Summers and D.H Putnam, eds, Irrigated alfalfa management for Mediterranean and Desert zones. Chapter 13: Oakland: University of California Agriculture and Natural Resources Publication. 8299. pp 12

Platt, T. 2005. Alfalfa’s Potential in Dryland Crop Production. Agricultural Horizons.April, p. 3. To purchase print or efile, go to:
http://pnw-ag.wsu.edu/AgHorizons/newsletter/2005/apr05.pdf

Thiébeau P, Parnaudeau V, Guy P (2003). Quel avenir pour la luzerne en France et en Europe ?. Courrier de l’environnement de l’INRA n°49, juin 2003. 29-46

Undersander D.J, Cosgrove D, Cullen E, Grau c, Rice M.E, Renz M, Sheaffer C, Shewmaker G, Sulc M (2011). Alfalfa Management Guide. American Society of Agronomy : Crop Science Society of America: Soil Science Society of America, 2011. To purchase print or efile, go to:
learningstore.uwex.edu/Alfalfa-Management-Guide-P1047.aspx] 64 p.