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Improvement of native perennial forage plants for sustainability of Mediterraean farming systems. Lucerne (*Medicago sativa*) breeding work in south Tunisia

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SUMMARY – Lucerne (*Medicago sativa*) is the most important fodder crop in the oases of Tunisia. A total of 20 accessions from representative '*Gabssia'* landraces were collected during the growing season of 2004 and evaluated *ex situ* on IRA experimental fields in Médenine and Gabes. Considerable variation was observed for all the characters studied according to the descriptors published by CEC and IBPGR (1984). For most variables, in particular yield, a significant difference was obtained between accessions. A new breeding programme was initiated in IRA (Institut des Régions Arides) within the PERMED project - PL 509140, 2004. Ideotypes collected from different oasis environments were used for plant breeding and selection of new varieties. A selection method was developed to select alfalfa for drought resistance in IRA Gabes and for salt resistance in IRA Médenine.

Key words: Evaluation, lucerne, *Medicago sativa*, oasis, Tunisia.

RESUME – *"Amélioration de plantes fourragères pérennes autochtones pour la durabilité des systèmes agricoles méditerranéens. Le travail de sélection sur la luzerne (*Medicago sativa*) dans le sud de la Tunisie". La luzerne (*Medicago sativa*) est le fourrage vert le plus important dans les oasis de la Tunisie. Un total de 20 accessions représentant les idéotypes locaux '*Gabssia*' a été rassemblé pendant la saison de culture (2004) et a été évalué* ex situ *dans les parcelles expérimentales de l'IRA de Médenine et Gabès. On a observé une considérable variation entre les accessions collectées pour tous les caractères étudiés selon les descripteurs édités par CEC et IBPGR (1984). Pour la plupart des variables, en particulier le rendement, une différence significative a été obtenue entre les accessions. Un nouveau programme de sélection variétale a été lancé à l'IRA (Institut des Régions Arides) dans le cadre du projet (PERMED - PL 509140, 2004) pour l'obtention de nouvelles variétés. La méthode de sélection a été développée pour choisir une luzerne plus résistante à la sécheresse pour les oasis de Gabès et plus résistante aux eaux saumâtres de la région de Médenine.*

Mots-clés : *Evaluation, luzerne,* Medicago sativa*, oasis, Tunisie.*

Introduction

Lucerne (alfalfa) is the most important sown perennial fodder in Tunisia. It is widely adapted and can be found through out the country. It is grown over 12, 410 ha (M.A.R.H, 2002, M.E.D.D, 2007). Lucerne has been grown in oases of Tunisia since many years and is currently grown on more than 9,720 ha across Southern Tunisia (ODS, 2005). As well as being a valuable fodder and forage crop (Dollé, 1990; Ferry and Toutain, 1990; Lasram, 1990; Tisserand, 1990), it is highly salt tolerant (Janati 1990; Mezni *et al*., 2002; Aganga and Tshwenyane, 2003).

Local landrace called '*Gabssia*' has a long history of cultivation worldwide and it has shown wide adaptation to a range of environments (Bolaños *et al*., 2000). Germplasm collection missions to all oases of Southern Tunisia were undertaken in 2004 and twenty accessions were collected (Table 1). This germplasm of arid regions is considered as an important genetic source for drought and salinity resistance for future breeding (Skourie, 1988; Leberre and Ramousse, 2003; PERMED, 2004).

"*In two countries, namely Tunisia and Morocco, alfalfa plant breeding exists with elite material, but cultivar development has not progressed over the last few years for diverse reasons. Two programs*

will be reinforced by PERMED through involvement in Workpackages 1 and 7 (PERMED Project).... New breeding programs will be initiated in IRA (Institut des Régions Arides) within the project. Results from WP1 (Workpackage 1), ideotypes collected from different oasis environments, will be used for Plant breeding and selection of new varieties" (PERMED project - PL 509140, 2004).

Table 1. '*Gabssia'* Lucerne accessions collected from the Oases of Southern Tunisia

Accessions numbers	Oases	Oases type	Altitude (m) a.s.l*
9	Gabes	Coastal	0 to 10
4	Tozeur	Continental	-20 to - 15
7	Kébili	Continental	-20 to - 15

*a.s.l: above sea level.

Material and methods

A total of 20 accessions (Table 1) from representative of '*Gabssia'* landraces were collected and evaluated *ex situ* at IRA experimental fields of Gabes and Médenine.

The Gabes site is generally characterised by oasis environment whereas Médenine trial have arid condition (Fig. 1). Basic information about the trial sites is presented in (Table 2).

Fig. 1. Collecting regions (Gabes, Kébili and Tozeur) of local Lucerne and IRA's experimental fields.

Table 2. Trial site characteristics in El Fjé (Médenine) and Chott el ferique (Gabes)

Locations	Sites	Mean t.min ℃ (winter)	Mean t.max ℃ (summer)	Mean rainfall	Soil	Water quality
Médenine	El fjet	10	30	150 mm	Sandy	Tap water
Gabes	Chott el ferique	12	28	182 mm	Sandy and silty	3-4 g/l

'*Gabssia*' ideotypes have become more numerous in recent years. However, according to farmers, the ideal plant morphology of '*Gabssia*' should be with high vigour, made of high number of stem and leaves, hollow (empty) stem, tolerate early cutting, semi to non dormant, and cutting regime about 8 to 10 cuts/year. Leaflets are elongate with laciniate top margin. Flowers mostly are violet and seeds colour is yellow.

Accessions were scored on different morpho-agronomic characteristics according to the descriptors for forage legumes published for CEC and IBPGR (1984).

Results

Considerable diversity was observed for all characters studied at IRA's experimental field.

The period from sowing to flowering varied from 59 to 62 days in the arid condition of Médenine and from, 93 to 100 days (in Gabes). About 88.8% of plants have blue-violet flower (light 45.8% and dark 43%). Vegetative growth habit shows a predominance of prostrate (73.9%) and only 7.8% of plants are erect. Leaf shapes are elongated (50%), ovate (47.5%) and round (2.5%). Length of the longest stem at full flowering (head included; when fully expanded) ranged from 29.33 cm to 66.68 cm, with a mean of 48.24 cm. Natural height three weeks after 1st cut ranged from 15.75 cm to 33.57 cm with a mean of 20.78 cm. The natural height three weeks after 2nd cut is range from 15.33 cm to 28.87 cm with a mean of 22.16 cm.

Calculated values of $F_{0.05}$ show that for all characters studied, inter-accessions differences are highly significant, therefore the accessions studied here are considered to be statistically different.

The information collected from the field evaluation and characterisation will make this collection a considerable resource for future work (Trommetter, 2000; Baudoin, 2001). The results have shown the type of germplasm that is suitable for each site and future work will more closely target this germplasm for breeding for quality and tolerance to stresses (specifically salinity and drought). The stress factors, especially drought and salinity, negatively effect plant growth and development (El-sharkwy, 1990).

To get high yield of agricultural crops under stress, tolerant varieties are needed. So, there is sufficient variation in local lucerne accessions for drought and salinity tolerant to be increased substantially by selection and breeding (Downes, 2002).

For salt tolerance, accessions will be subject to a high saline environment (water of irrigation with high salinity) and plants that survive and produce important economic field's yields are considered tolerant. Those plants will be used to develop *inbred lines*. During each generation of inbreeding, we select only vigorous and high salt-tolerant plants. Inbred lines will be crossed to develop new *synthetic cultivar* "tolerant to water salinity, drought and heat" (Fig. 2).

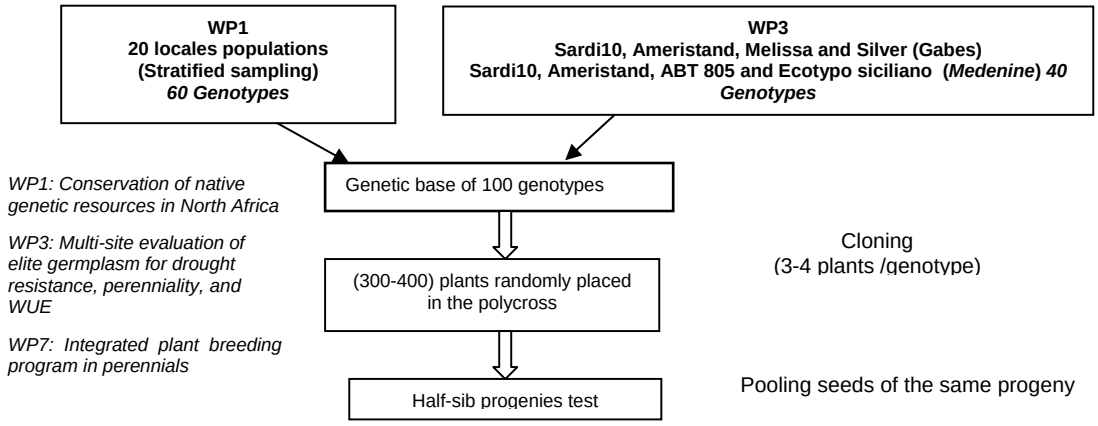

Fig. 2. Breeding design to select synthetic varieties for arid regions of Tunisia proposed by Dr. Paolo Annicchiarico and Dr. Luciano Pecetti.

(i) Choice of the genetic base. We include some germplasm derived from the best-performing foreign varieties in Gabes and Médenine WP 3 trials, considering the excellent response of some of these varieties in comparison with the local *'Gabssia*' and the other north-African cultivars. We include about 40% of the total genotypes forming the genetic base from these varieties. We may include in Gabes: Sardi 10, Ameristand, Melissa and Siriver and in Médenine: Sardi 10, Ameristand, ABT 805 and Ecotipo siciliano based on the variety responses over the second year at the sites.

(ii) Obtainment of the genotypes for the genetic base. For each site and foreign variety, we suggest to obtain the 10 genotypes by digging out 10 surviving, best-looking plants from the border area of WP 3 plots (2 or 3 plants for each of the four replicates of the variety). Likewise, we take the 60 genotypes of the local genetic base from best-looking, surviving.

(iii) Parent selection from the genetic base. We suggest a genetic base of about 100 genotypes per site, because we feel that this number may allow us to select the parent genotypes for the future variety directly on the basis of progeny testing (avoiding the clonal selection stage). We also recommend to finally select not less than 10-12 parent genotypes for the variety (to avoid inbreeding and narrowing of the genetic base). We should use cloning just to obtain replicates of each genotype for the polycross, e.g. 3-4 plants, for a total of 300-400 plants randomly placed in the polycross, pooling the seed of the same genotype at harvest for the following half-sib progeny testing.

Propagation by cuttings of Lucerne

Lucerne can be stem propagated without addition of hormones, as long as, the cuttings are taken from the upper part of the stem, and they are maintained in humid environment (INRA, 2001; Hood and Howard, 2002; M.M. Tavares de Sousa, pers.comm).

Best-looking plants were selected for cuttings. After stem propagation in the greenhouse, cuttings were transplanted to the field for acclimatization on 4 August at IRA experimental field.

Table 4. Results of cuttings

Varieties	Success rate of		
	Cutting	Transplantation	Acclimatization
Ecotipo siciliano	95.0%	71.5%	79.6%
Ameristand	60.0%	78.3%	48.5%
Sardi 10	65.0%	89.2%	67.3%
Local landrace 'Gabssia'	62.0%	58.0%	73.0%

The best results were obtained with Ecotipo siciliano for all actions (Success rate of cutting=95%, tansplantation= 71,57% and Acclimatization=79,62%) followed by local landraces 'Gabssia'.

Conclusions

Lucerne has been grown in oases of Tunisia since many years. It is highly salt tolerant and is widely adapted to southern Tunisia farming regions (oases).

With the support of PERMED Project, Germplasm collection missions to all oases of Southern Tunisia were undertaken and twenty accessions were collected. Accessions were scored on different morpho-agronomic characteristics according to the descriptors for forage legumes published for CEC and IBPGR (1984). Considerable diversity was observed for all characters studied at IRA's experimental field.

A new breeding program is initiated in IRA within the project. Ideotypes collected from different oasis environments, are used for plant breeding and selection of new varieties. Selection methods are developed to select alfalfa for drought resistance in IRA Gabes and for salt resistance in IRA Médenine.

A genetic base of about 100 genotypes per site was used to constitute parent selection.

The genetic base was included some germplasm derived from the best-performing foreign varieties in Gabes and Medenine WP 3 trials. We include about 40% of the total genotypes forming the genetic base from these varieties. In Gabes: Sardi 10, Ameristand, Melissa and Siriver, and in Médenine: Sardi 10, Ameristand, ABT 805 and Ecotipo siciliano.L.

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