Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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Asian Journal of Plant Sciences 5 (6): 996-1001, 2006
ISSN 1682-3974

Genetic Diversity and Taxonomic Status of *Lotus glaber* Mill populations in Fayoum Depression

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Abstract: This study was applied to elucidate the taxonomic status and to evaluate genetic diversity of *L. glaber* Mill. populations distributed along 5 territories of Fayoum depression. This was carried out through scoring of 39 morphological characters, chromosome counts and isozyme electrophoresis. The diagnostic morphological characters and diploid chromosome number 2n = 12 elucidated that the collected accessions from Fayoum depression should be treated as *L. glaber*. Populations having higher genetic diversity measures were grouped in one cluster. These populations were collected from highly saline soils with imperfect to poor drainage insuring the tolerance of *L. glaber* to salinity and poor soil conditions. This verified the importance of *L. glaber* as a valuable genetic resource that can be used for improvement of *L. corniculatus*. More research was initiated in our lab to link salt tolerance trait with DNA marker.

Key words: *Lotus glaber*, genetic diversity, isozymes, populations

INTRODUCTION

The genus *Lotus* L. is an important forage legume that is found in both Old and New world. The Old World species are distributed around the Mediterranean, up to the Arctic and down to the Nile through Ethiopia and East Africa to South Africa and Western Asia (Tutin *et al*., 1980; Zohary, 1966; El-Hadidi and Fayed, 1994/1995; Boulos, 1999; Jafri, 1980). Many of the species that comprise the genus *Lotus* are closely related and constitute complexes that have been grouped by similar morphological characteristics (Kirkbride, 1999). Among these complexes, *L. corniculatus* group encompass 11 diploid (2n = 2x = 12) species in addition to *L. corniculatus* L. as the only tetraploid (Grant and Small, 1996; Grant, 1999). The nature of tetraploidy of *L. corniculatus* and its phylogenetic relationships with the diploid species in *L. corniculatus* group has been long debated (Ross and Jones, 1985; Raelson and Grant, 1988; Grant and Small, 1996; Steiner 1999). Several authors have evidenced the autotetraploid origin of *L. corniculatus* and that *L. glaber* Mill is one of its direct progenitors (Grant and Small, 1996; Grant, 1999; Arambarri, 2000; Fjellstrom *et al*., 2001; Beuselinck *et al*., 2003; Bardini *et al*., 2004).

In Egypt, the typical *L. corniculatus* has not been recorded and the taxon recorded by Boulos (1999) was identified as *L. corniculatus* var. *tenuifolius* L. This was confirmed by El Hadidy (2003) using diagnostic characters of the style and corolla lengths and was treated as *L. glaber*. This species is distributed along the Nile Valley and Nile Delta, Fayoum depression, very rare in deserts (El-Hadidi and Fayed, 1994/1995; Boulos, 1999; El Hadidy 2003).

The Fayoum area (~1700 km^2) occupies a circular deep depression in limestone plateau at the northern part of the Western Desert of Egypt between longitudes 30°23′ and 30°5′E and latitude 29°5′ and 29°35′N. The surface of the depression slopes from +40 m at the southern east edge towards the lowest part of about -45 m at the northern west where Lake Qarun (Fig. 1). The main human activity in Fayoum depression is cultivation. The cultivated land receives irrigation water from a network of irrigation canals radiated from Bahr Yousef, which receives fresh water from the Nile. During summer, the main flow is insufficient especially in downstream areas. Pumping of drainage water into fresh water canals was applied to compensate such water shortage, which raised soil salinity (Abd-El-Motaleb, 1997).

A common problem with the improvement of many forage species including *L. corniculatus* has been the lack of genetic variability and the continued cultivars selection from a narrow germplasm base (Steiner, 1999; Steiner *et al*., 2001). In order to broaden *L. corniculatus* genetic base, it is necessary to study genetic diversity that may be present in its wild progenitors as a valuable genetic resource. *L. glaber* is a species adapted to tolerate salinity, infertile soils and long flooding conditions more

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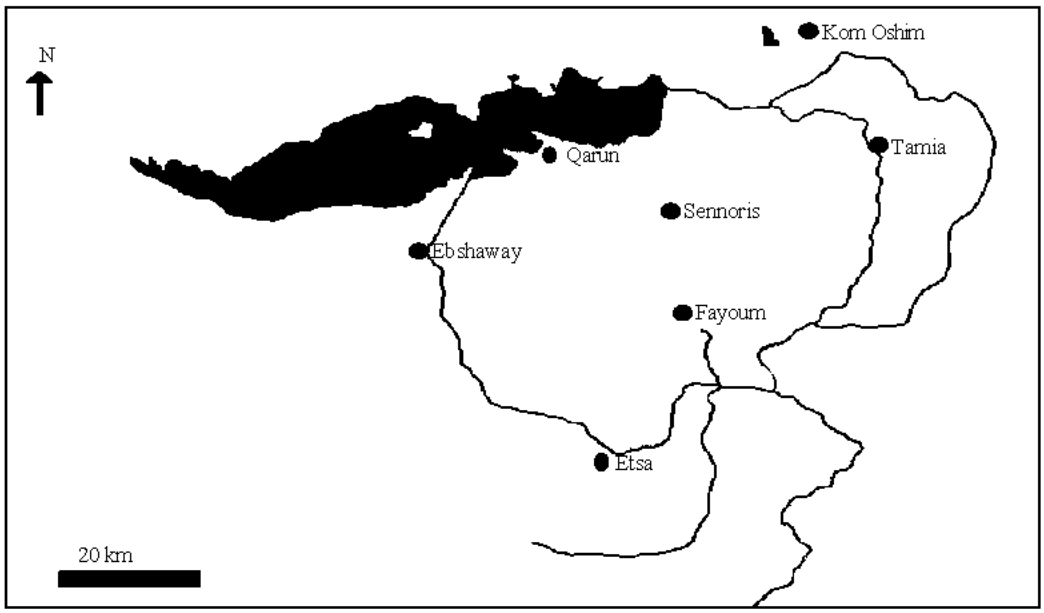

Fig. 1: Location of the studied populations of *L. glaber* collected from Fayoum depression

than *L. corniculatus* (Maceira *et al*., 1999; Blumenthal and McGraw, 1999; Striker *et al*., 2005; Banuelos and Beuselinck, 2003).

The present investigation was carried out to elucidate the taxonomic status of *L. glaber* in Fayoum depression through morphological description and chromosome count in addition to evaluating genetic diversity among populations of this taxon using isoenzyme electrophoresis.

MATERIALS AND METHODS

Plant material: A total of 90 accessions were collected to represent 7 *L. glaber* populations that are distributed in the 5 territories of Fayoum depression. The details of the locations and their description are shown in Fig. 1 and Table 1. For the morphological description of this species, 39 morphological characters were applied that include stem (4 characters), leaflets (12), inflorescence (2), flower (6), pod (3) and seed (2).

Seed germination: Seeds were surface sterilized by soaking in 70% (v/v) ethanol for 1 min, then rinsed several times with sterile distilled water. The seeds (25 seeds) were germinated for 7 days at 25°C in sterilized petri dishes with three moist filter papers.

Chromosome count: Root tips were soaked in 0.003% colchecine for 4 h then fixed in ethanol:glacial acetic acid (3:1 v/v) for 2 h and stained in HCl carmine for the appropriate time required for the root tips to become dark red. The deeply stained root tips were macerated with 45% glacial acetic acid on clean slides. Cells with well-spread metaphase chromosomes were count and photographed.

Isoenzyme analysis:

Extraction: Green leaves of 7-day-old seedlings were macerated in 5 mL saline solution (0.8% NaCl, 0.2% $NaNO_3$, 10% glycerol and 1% bromophenol blue) then centrifuged at 10000 g for 15 min. Supernatants were collected in pre-chilled tubes and stored at -20°C until use for electrophoretic separation of isoenzymes.

Electrophoresis: The isoenzyme separation was carried out using mini vertical slabs of 7.5% acrylamide concentration according to Laemmli (1970). Aliquots (20 μL) of extracts loaded onto the gels. Electrophoresis was carried out at 20 mA for 60 min. The gels were stained for 4 isoenzyme systems (acid phosphatase, amylase, esterase and malate dehydrogenase) according to Eduardo Vallejos (1983).

Data analysis: For each enzyme, gene loci and alleles were inferred and labelled following numerical and alphabetical sequence, respectively. Elementary descriptors of isozyme variation (allele frequency, number of alleles per locus (A), percentage of polymorphic loci (P) and expected heterozygosity (H)) and genetic distances (D) (Nei, 1978)

Table 1: The studied locations and sites from which *Lotus glaber* were collected

Location	site	Population code	Elevation	Soil texture	ECe	Salinity	Drainage	No. of accessions
Kom Oshim (70 km South to Cairo)	Reclaimed land	Kom	40	Sand	25.6	Moderate	Excessive	15
Tamia	Irrigated fields	Tam	-13	Clay	6.7	Non	Well	12
Sennuris	Irrigated fields	Sen	-4	Sandy clay	10.7	Non to moderate	Imperfect	13
Ebshaway	Irrigated fields	Ebs	-9	Silty loam	62.5	High	Poor	12
Qaroun	Irrigated fields	Qar	-35	Loamy sand	159.2	Very high	Poor	8
Fayoum	Irrigated fields	Fay	17	Clay	23.6	Moderate	Imperfect	14
Etsa	Irrigated fields	Ets	18	Loamy sand	4.26	Non	Well	16

Data on elevations, soil texture, salinity and drainage were adopted from Abd-El-Motaleb (1997 and 2002)

were calculated using PopGen32 software (Yeh *et al*., 1999). Values of Nei's genetic distance were used for clustering of the collected populations using unweighted pair-group method, arithmetic average (UPGMA) (Sneath and Sokal, 1973) using MINITAB-13 for windows (Minitab Inc., 2000).

RESULTS

Morphological description:

Synonyms:

Lotus corniculatus subsp. *tenuifolius* (L.) P. Fourn., Quatre Fl. France: 564.1953.

Lotus corniculatus subsp.*tenuis* (Willd.) Berher in Louis, Dep.Vosges 2:72, 1887.

Lotus noeanus Boiss.,Diagn.Pl.Orient.ser.2,2:21.1856

Lotus tenuifolius Reichenb., Fl. Germ. Excurs.: 506.1832 [non Burm. fil. cap. prodr.: 22.1768 [nom. Illeg.].

Lotus tenuis Willd., Enum.Pl.Hort.Berol.:797.1809.

Perennial herbs, decumbent or ascending, stem glabrous with extremely variable stem branching (5 to more than 20 branches) and length (7-60) cm). Leaf compound of 5 glabrous or hairy leaflets, upper 3 leaflets oblanceolate to elliptic or obovate with acute to acuminate apex; the central leaflet length 7-11 mm long and width 2-4 mm; the peripheral leaflets measured 7-10 mm long and 2.0-2.7 mm width; lower pair of leaflets lanceolate to ovate, with acute to acuminate apex, slightly shorter than upper leaflets. Flowers axillary 2 to 3 forming an umbel, bright yellow, sometimes flushed with red, pink, orange or green veins; 7.5-8.5 mm long, about twice as long as the calyx. Axillary peduncle much longer than the subtending leaf, 4 to >6 mm long. Bract sessile of 3-leaflets, the 2 peripheral equal, 3-6 mm long, the central longer, 4-7 mm long. Pedicel very short and straight about 1.25-1.75 mm long. Calyx glabrous with sub-equal teeth nearly equal to the calyx tube. Corolla 7.5-8.5 mm long, about twice as long as the calyx. Standard suborbicular, longer than the keel and wings. Keel tip incurved, yellow with long beak. Wings broadly obvate, with obtuse apex. Style length 4.4-56.0 mm. Pod straight, linear, broad, 2.5-4.0 cm long, usually tipped with the persistent style base; 7-20 seeded; valves brown. Seed rounded with smooth brown to dark brown test.

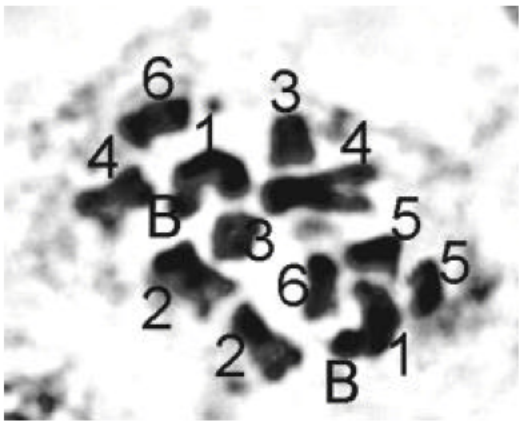

Fig. 2: Metaphase plate of *L. glaber* accession collected from Qarun with chromosome number 2n = 12+2B

Chromosome count: The chromosome set of all studied accessions comprised of 12 chromosomes. Populations of Qarun and Ebshawai were characterized by two supernumerary (B) chromosomes (Fig. 2).

Isoenzyme analysis: A total of 14 loci were recorded collectively all over the studied populations (Table 2). The population of Etsa was characterized by the absence of the locus *Aph*1 while that of Tamia was distinguished by the absence of loci *Amy*4, *Aph*2, *Est*1, *Est*3 and *Mdh*2. Both populations shared the absence of loci *Amy*1 and *Est*3. At the loci *Amy*3, *Aph*3, *Est*1 and *Mdh*3 some populations exhibited the mono-allelic expression while remaining populations exhibited the bi-allelic expression of these loci (for example, the populations of Ebshaway, Tamia and Etsa at the locus *ApH*3, Table 3). At the remaining loci, the studied populations varied in the allele that having the higher frequency (the predominant allele), others showed a considerable balance in the frequency of the two alleles of the same locus.

Table 2: Allele frequency at all observed isozyme loci of the collected poulations of *L. glaber*

Locus	Allele	1 Qarun	2 Fayoum	3 Sennoris	4 Ebshawai	5 Tamia	6 Kom Oshim	7 Etsa
Amy1	A	1.000	1.000	1.000	1.000	0.000	1.000	0.000
Amy2	A	1.000	0.667	1.000	1.000	1.000	1.000	1.000
	B	0.000	0.333	0.000	0.000	0.000	0.000	0.000
Amy3	A	0.250	0.133	0.143	0.000	0.000	0.143	0.000
	B	0.750	0.867	0.857	1.000	1.000	0.857	1.000
Amy4	A	1.000	1.000	1.000	1.000	0.000	0.000	1.000
	B	0.000	0.000	0.000	0.000	0.000	1.000	0.000
Aph1	A	0.750	0.313	0.300	0.125	0.000	0.250	0.000
	B	0.250	0.688	0.700	0.875	1.000	0.750	0.000
Aph2	A	0.667	0.417	0.600	0.500	0.000	0.667	0.000
	B	0.333	0.583	0.400	0.500	0.000	0.333	1.000
Aph3	A	0.833	0.750	0.667	1.000	0.500	0.750	0.000
	B	0.167	0.250	0.333	0.000	0.500	0.250	1.000
Est1	A	0.500	0.300	0.000	0.000	0.000	0.000	0.000
	B	0.500	0.700	1.000	1.000	0.000	1.000	1.000
Est2	A	0.417	0.250	0.688	0.500	0.000	0.167	0.375
	B	0.583	0.750	0.313	0.500	1.000	0.833	0.625
Est3	A	0.000	0.667	0.000	0.000	0.000	0.000	0.000
	B	1.000	0.333	1.000	1.000	0.000	1.000	0.000
Mdh1	A	0.375	0.429	0.333	0.500	0.500	0.500	0.500
	B	0.625	0.571	0.667	0.500	0.500	0.500	0.500
Mdh2	A	0.667	0.546	0.375	0.833	0.000	0.833	0.000
	B	0.333	0.455	0.625	0.167	0.000	0.167	1.000
Mdh3	A	0.286	0.091	0.167	0.000	0.000	0.200	0.000
	B	0.714	0.909	0.833	1.000	1.000	0.800	1.000
Mdh4	A	0.500	0.692	0.500	0.600	0.000	0.500	0.333
	B	0.357	0.077	0.375	0.100	0.000	0.250	0.667
	C	0.143	0.231	0.125	0.300	1.000	0.250	0.000

Table 3: Genetic diversity measures of the collected populations of *L. glaber*

Population	A	P (%)	Hexp
Kom Oshim	1.714	60.00	0.246
Tamya	1.250	13.33	0.125
Sennoures	1.714	60.00	0.272
Ebshawai	1.500	40.00	0.181
Fayoum	1.929	80.00	0.344
Etsa	1.271	20.00	0.129
Qaroun	1.786	66.67	0.313
L. glaber	2.000	86.67	0.372

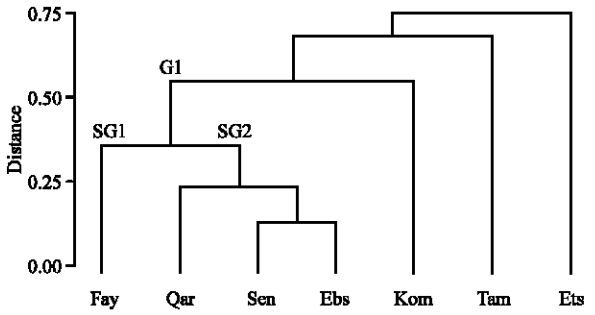

Fig. 3: UPGMA clustering of the studied population of *L. glaber* based on Nei's genetic distance

The highest values of mean alleles per locus (A), percentage of polymorphic loci (P) and theoretical heterozygosity (H) were observed for the population of Fayoum (A = 1.929, P = 80%, H = 0.344; Table 3) followed by those collected from Qarun (A = 1.786, P = 66.67%, H = 0.313, Table 3), then Sennoris and Kom Oshim (similar values of A (1.714) and P (60%), H = 0.272 and 0.246, respectively), Ebshaway (A = 1.5, P = 40%, H = 0.181). The lowest values of the three genetic diversity measures were observed for the populations of Etsa followed by Tamia. The UPGMA clustering based on Nei's genetic distance grouped the populations of Fayoum, Qarun, Sennoris and Ebshaway at distance 0.35 (G1, Fig. 3) while each of the remaining populations comprised a group of its own. At distance 0.25, the population of Fayoum was separated in a single subgroup (SG1, Fig. 3) while those of Qarun, Sennoris and Ebshaway comprised another subgroup (SG2, Fig. 3).

DISCUSSION

Knowledge of available plant genetic resources is fundamental to support programs for the development of new cultivars, as well as to protect existing natural resources. The assessment of genetic diversity should be based on sound taxonomic identification. The investigated accessions of all populations were observed to have short style (4.0-4.56 mm), short corolla (7.5-8.5 mm) and central leaflets 2.0-3.5 times longer than wide. Comparing these values with those of *L. corniculatus* (style 5-7 mm, corolla 10-18, central leaflet 1.5-3 times longer than wide; Kirkbride, 1999; El Hadidy, 2003) elucidated that the collected accessions from Fayoum depression should be treated as *L. glaber*.

The chromosome counts showed that all the studied accessions are diploid with 2n = 2x = 12. Several authors

have affirmed the diploid conditions of *L. glaber* that is essential for its discrimination from the tetraploid *L. corniculation* (Raelson and Grant, 1988; Grant and Small, 1996; Grant, 1999; Steiner, 1999; Fjellstrom *et al*., 2001; Beuselinck *et al*., 2003). This added cumulative evidence in favour of *L. glaber*.

The remarkable feature of the metaphase plate in the present investigation was the occurrence of B chromosomes in accessions collected from Qarun and Ebshawai. The occurrence of B chromosomes (2n = 12 + 1B) was previously observed in some Egyptian accessions of *L. corniculatus* (now *L. glaber*) that doubled the quantities of seed proteins compared to those lacking B. (Sammour *et al*., 1991). It was also found that drought increased the proportion of survivors of *Allium schoenoprasum* with B chromosomes (Bougourd and Plowman, 1996). The relation between B chromosomes and environmental impact is not clear (Puertas, 2002). However, it could be possible that the occurrence of B chromosomes might help populations of Qarun and Ebshawai to tolerate highly saline soils.

The populations of Etsa, Tamia and Kom Oshim were characterized by relatively lower values of genetic diversity measures (Table 3). They were also separated in distinctive groups when UPGMA clustering based on Nei's genetic distance was applied (Fig. 2). These populations were distinguished by their non- to moderately-saline soils with well to excessive drainage (Table 1). On the other hand, the populations collected from Fayoum, Qarun, Sennoris and Ebshaway showed the highest values of genetic diversity measures and were grouped in a single subgroup according to Nei's genetic distance (SG1, Fig. 3). These populations were collected from saline to very highly saline soils with imperfect to poor drainage (Table 1). These findings are consistent with the previous observations that *L. glaber* is adopted to tolerate salinity and flooding (Blumenthal and McGraw, 1999; Maceira *et al*., 1999; Banuelos and Beuselinck, 2003; Striker *et al*., 2005; Striker *et al*., 2006). Since the response of a species to challenge an environmental stress correlates with its genetic variation, the populations of Qarun, Fayoum, Sennoris and Ebshaway provides a valuable genetic resource that can be used for the improvement of *L. corniculatus*.

In conclusion, the taxon collected from Fayoum depression was identified as the diploid *L. glaber* that was observed to tolerate salinity and flooding. The salt tolerant *L. glaber* populations were those characterized by higher genetic diversity and can be introduced for gene banks as valuable genetic resource. More research was initiated in our lab using DNA fingerprinting to find out DNA marker(s) that can be linked with salt and flooding tolerance of *L. glaber*.

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