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Karyotypic study of some Iranian species and populations of *Lotus* L.

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A karyotypic study of 13 populations belonging to 7 Iranian *Lotus* species was performed for the first time. The study showed x = 6 and 7 are available in Iranian *Lotus* species; all taxa studied except *L. corniculatus* (4x) were diploid. The species studied varied in their karyotypic formulae and symmetry. They also differed significantly in their total chromatin length as well as size of long arms and short arms, indicating the occurrence of both structural and quantitative changes in their karyotypes during the species diversification. Clustering of the *Lotus* species based on karyotypic features partly supports their taxonomic treatment.

Key words: Lotus, karyotype, taxonomy, species diversification

Introduction

The genus *Lotus* L. (tribe Loteae, Leguminosae) comprises about 100 annual and perennial species growing widely throughout the world (POLHILL 1981, 1994a, b). *Lotus* species are mainly distributed in the Mediterranean and NW of America (POLHILL and RAVEN 1981). There is discrepancy about the number of *Lotus* species growing in Iran, for example according to MOUSAVI (1974) ten species grow in Iran while PARSA (1948) and CHRTKOVA-ZERTOVA (1982) reports the occurrence of only 9 species. Some of the *Lotus* species are among important forage plants of Iran such as: *L. corniculatus, L. tenuis, L. pedunculatus* and *L. angustissimus*.

Although there have been extensive reports on the biosystematic studies of the *Lotus* species from the other parts of the world (see for example GRANT 1995), there are only limited studies on the genus *Lotus* of Iran reporting seed proteins and meiotic chromosome pairing in some of the species only (JALILIAN et al. 2005, SHEIDAI and JALILIAN 2006). The present study was performed as a part of a biosystematic study of the genus *Lotus* in Iran, reporting karyotypic features in 12 populations of 7 species for the first time trying to illustrate the role of cytological changes in the species diversification.

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Materials and methods

Plant material

Cytological studies were performed in 13 populations of 7 *Lotus* species namely: 1 – *L. corniculatus* L. (three populations), 2 – *L. garcinii* DC, 3 – *L. gebelia* Vent. (three populations), 4 – *L. laricus* Rech. f., 5 – *L. schimperi* Steud (two populations), 6 – *L. tenuis* Waldst. et Serg. (two populations) and 8 – *L. angustissimus* L. (Tab. 1). The voucher specimens are deposited in the herbarium of Shahid Beheshti University (HSBU) and TARI.

Cytological study

For cytological studies freshly grown root tips were collected from the seeds of at least 10 randomly selected plants in each species, pretreated with 0.002 mol 8-hydroxyquinolin (1–2 hrs.) and fixed in ethanol: acetic acid (3:1) for 24 hrs., then washed thoroughly and macerated in 1N HCl for about 30 sec. at n 60 °C. Squash technique was used for cytological studies with 2% aqueous aceo-orcein as the stain.

The somatic chromosome number and karyotypic details were studied in at least 5 well prepared metaphase plates. The chromosomes were sketched with the use of camera lucida and measurements performed accordingly from such sketches.

The chromosomes were identified according to LEVAN et al. (1964), karyotype symmetry was determined according to STEBBINS (1971) and ROMERO-ZARCO (1986), while other karyotypic parameters such as total form percentage (TF) %, S% and difference of range of relative length (DRL) (HUZIWARA 1962) and coefficient of variation (CV) were also determined (SHEIDAI et al. 2000).

The analysis of variance test (ANOVA) was performed to reveal a significant difference in the size of chromosomes among the populations of each species as well as among species having similar somatic chromosome numbers (SHEIDAI et al. 2000). In order to group the species with similar somatic chromosome number, having similarity in their karyotypic features, cluster analysis and ordination based on principal components (PCA) and principal coordinate analysis (PCO) were performed (SHEIDAI et al. 2000). The statistical analyses were performed with SPSS ver. 9 (1988) and NTSYS ver. 2.02 (1988).

Results

We found the chromosome numbers and morphometry in *Lotus glaber* and *L. angustissimus* (2n = 2x = 12); L. *garcinii*, *L. gebelia*, *L. laricus*, and *L. schimperi* (2n = 2x = 14) and *L. corniculatus* (2n = 4x = 24) (Tab. 1, Figs. 1–4). In *Lotus* species the length of the shortest chromosomes varied from 6.96 μm in the Jalaei population of *L. schimperi* to 18.01 μm in the Mehregan population of *L. corniculatus*, the length of longest chromosomes also varied from 8.22 μm in the Jalaei population of *L. schimperi* to 23.71 μm in the Mehregan population of *L. corniculatus*.

Among the species having the 2n = 12 chromosome number, the highest total chromatin length was observed in the Khoramshar population of *L. glaber* (20.04 μm), while the lowest value occurred in *L. angustissimus* (16.69 μm). Among species having the 2n = 14 chromosome number, the highest total chromatin length was observed in *L. garcinii* (29.54 μm), while the lowest value occurred in the Jalaei population of *L. schimperi* (15.21 μm).

Tab. 1. Karyotypic details of *Lotus* species studied. Total chromatin length, L – length of the long arm, S – length of the short arm, ST – Stebbins class, KF – karyotype formulae.

Species	Locality	2n	TL	L	S	L/S	TF%	CV	S%	X	L-S	DRL	A_1	A_2	ST	KF
L. garcinii	Bandar Abbas	14	29.54	16.28	13.04	1.25	44.19	20.40	57.81	4.22	2.32	7.85	0.29	0.20	1A	7m
L. gebelia1	Kordestan Shafabaksh	14	18.83	11.10	7.61	1.45	40.81	18.11	57.48	2.69	1.42	7.54	0.31	0.18	1A	7m
L. gebelia2	Kordestan Nashoor	14	25.53	15.49	10.34	1.49	40.12	25.32	45.97	3.64	2.75	10.77	0.30	0.25	2B	5m + 2sm
L. gebelia3	Kordestan Sardasht	14	24.09	13.37	10.73	1.24	44.92	24.61	47.21	3.44	2.46	10.21	0.19	0.24	1B	7m
L. schimperi1	Hormozgan Hasanlangei	14	15.50	8.23	6.98	1.17	44.94	26.32	45.90	2.21	1.65	10.64	0.15	0.26	1B	1M + 6m
L. schimperi2	Hormozgan Jalaei	14	15.21	8.22	6.96	1.18	45.86	23.97	50.00	2.17	1.50	9.86	0.13	0.24	1B	3M + 4 m
L. laricus	Hormozgan Hasanlangei	14	16.69	9.10	7.57	1.20	44.67	14.37	65.81	4.39	1.88	11.27	0.20	0.14	1A	1M + 6m
L. corniculatus1	Kermanshah Sahneh	24	34.22	19.04	15.08	1.26	44.14	30.91	37.87	2.85	2.87	8.39	0.20	0.30	1B	4M + 4m + 1sm
L. corniculatus2	Kordestan Kilehneshin	24	28.25	15.10	13.05	1.15	46.42	29.84	38.66	2.35	2.30	8.14	0.13	0.29	1B	3M + 9 sm
L. corniculatus3	Kermanshah Mehran	24	41.75	23.71	18.01	1.31	42.97	28.66	40.54	3.47	3.30	7.91	0.24	0.28	2B	8m + 4 sm
L. tenuis 1	Ghooreigel Khoramshahr	12	20.04	11.22	8.97	1.25	45.73	28.98	45.20	3.34	2.63	13.13	0.19	0.28	1B	6m
L. angustissimus	Noshahr	12	16.69	9.10	7.57	1.20	45.79	15.21	65.15	2.78	1.15	6.89	0.16	0.15	1A	2M + 4m

Karyotypic formulae of the species studied show that most of chromosomes are of the metacentric type (M or m) with a few submetacentric chromosomes (sm). Karyotype symmetry of the *Lotus* species studied also show they mostly occupy the 1A, 1B and 2B classes of the STEBBINS classification, which are considered rather primitive classes in this system. By using ROMERO-ZARCO symmetry indices of A_1 and A_2 we can determine the more asymmetric karyotypes among species with similar STEBBINS symmetry classes. For example among the species with 1A class, the Shafabakhsh population of *L. gebelia* possesses the highest value for A_1 (0.31, therefore has a more asymmetric karyotype. Similarly among the species with the 1B symmetry class, the Sahneh population of *L. corniculatus* possessed the highest value for A_1 (0.20).

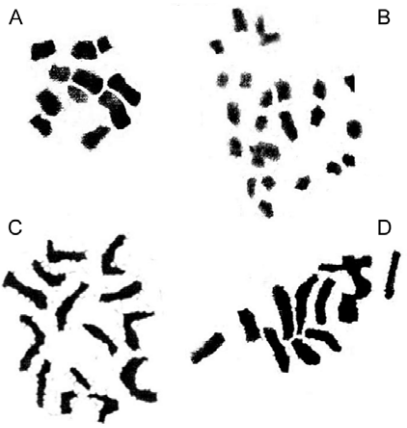

Fig. 1. Representative metaphase cells in *Lotus* species studied. A – *L. tenuis* Khoramshahr population (2n = 12), B – *L. corniculatus* Mehregan population (2n = 24), C – *L. garcinii* (2n = 14), D – *L. gebelia* Nashor population (2n = 14). Scale bar denotes 15 μm.

Three populations of *L. gebelia* occupied 3 different classes, 1A, 1B and 2B. Moreover ANOVA test showed a significant difference in total chromatin length and the length of short arms ($p < 0.01$) among these populations.

The tree populations of *L. corniculatus* studied also occupied the 1A and 1B classes of Stebbins system and also differed significantly in their total chromatin length and the length of the long and short arms ($p < 0.01$). However, two populations of *L. schimperi* and two populations of *L. glaber* occupy the 1B class of Stebbins but do not differ significantly in their total chromatin length as well as length of long arms and short arms ($p > 0.88$).

The species were grouped on the basis of their karyotypic similarity (relative karyotypic parameters in table 1) (Figs. 3, 4). Different clustering methods like those of UPGMA, WARD and the single linkage method produced similar results, of which UPGMA possessed the highest cophenetic correlation ($r = 0.74$) and is used for discussion. PCO ordination of similar data supported the clustering result.

Discussion

The chromosome numbers in *Lotus glaber* and *L. angustissimus* (2n = 2x = 12); L. *garcinii*, *L. gebelia*, *L. laricus*, and *L. schimperi* possessed (2n = 2x = 14) and *L. corniculatus* (2n = 4x = 24), where confirmed as in earlier studies (GRANT 1995, LÖVKVIST and HULTGÅRD 1999, SHEIDAI and JALILIAN 2006, SNOGERUP 1985, VIOQUE and PASTOR 1991, ZANDSTRA and GRANT 1968).

The present study shows that all the species studied are diploid except *L. corniculatus* which is tetraploid. Our previous meiotic study showed that different populations of *L. cor-*

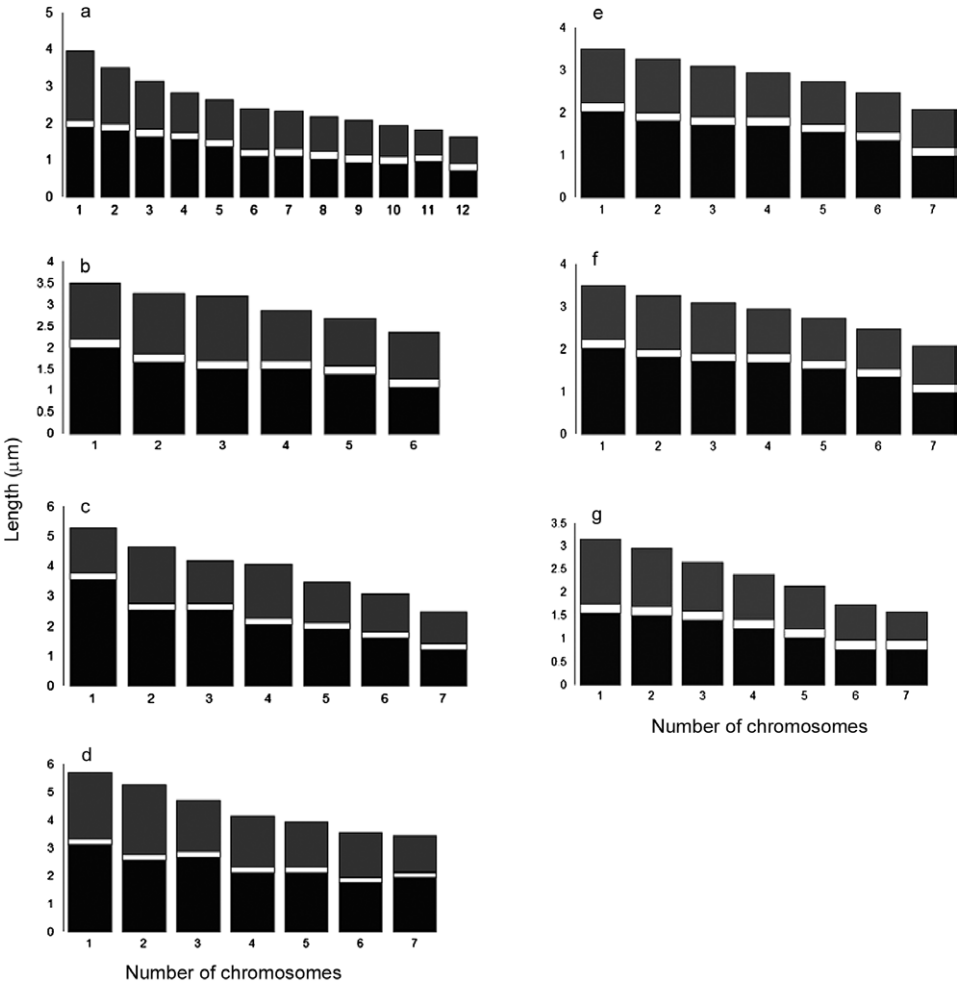

Fig. 2. Representative ideograms in *Lotus* species studied.

niculatus form only bivalents and no quadrivalents are formed in the metaphase of meiosis I (SHEIDAI and JALILIAN 2006), suggesting its allotetraploid nature. also reported mostly The occurrence of bivalents in the meiosis of *L. corniculatus* has been reported earlier (DAWSON (1941). The tetrasomic inheritance (FJELLSTROM et al. 2001), led STEBBINS to consider this species a segmental allopolyploid (GRANT 1995).

Basic chromosome numbers of x = 5, 6 and 7 have been reported in the genus *Lotus* (GRANT 1995) indicating the role of aneuploidy in the evolution of the genus. It is considered that evolution has proceeded in the genus *Lotus* by means of a descending series from an eight-chromosomed ancestor to 7, 6 and finally to 5 (GRANT 1995).

Based on morphological characters the *Lotus* species of Iran have been given different taxonomic treatments (for example PARSA 1948, CHRTKOVA-ZERTOVA 1982). The CHERTKOVA-ZERTOVA (1982) taxonomic treatment of the genus is the most recent and complete

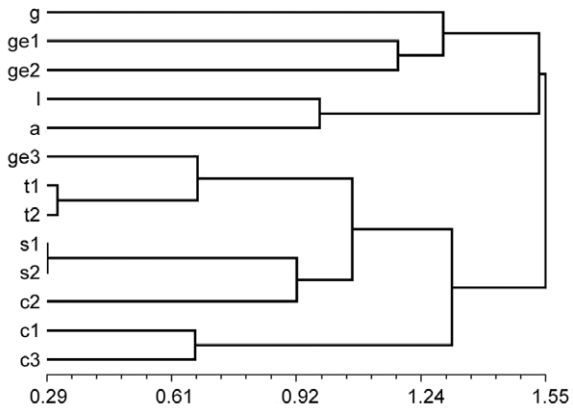

Fig. 3. UPGMA clustering of *Lotus* species studied. c1-3 – *L. corniculatus* (Sahneh, Kilehneshin and Mehran populations respectively); t1, t2 – *L. tenuis* (Ghooreigel and Khoramshahr populations respectively); ge1-3 – *L. gebelia* (Shafabaksh, Nashoor and Sardasht populations respectively); g – *L. garcinii*; l – *L. laricus*; a – *L. angustissimus*; s1 and 2 – *L. schimperi* (Hasanlangei and Jalaei populations respectively).

one, and has been supported by our morphometric and protein studies (JALILIAN et al. 2005, and unpublished data). They considers in total 10 *Lotus* species growing in Iran distributing them in four sections namely: 1 – Lotus, 2 – Loteae, 3 – Erythrolotus and 4 – Ononidium.

The first section is composed of *L. glaber, L. krylovii*, *L. corniculatus*, *L. angustissimus* (all having x = 6), *L. gebelia* and *L. micauxianus* (possessing x = 7).

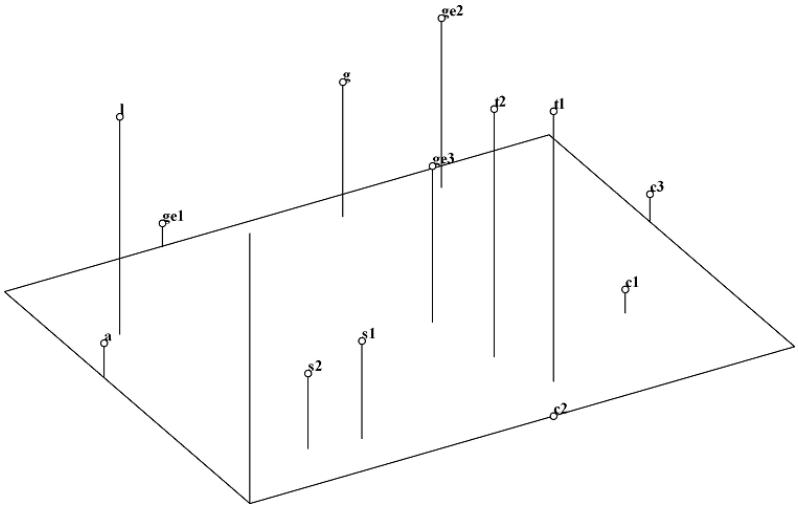

Fig. 4. PCO plot of *Lotus* species studied. c1-3 *L. corniculatus* (Sahneh, Kilehneshin and Mehran populations respectively); t 1 and t2 – *L. tenuis* (Ghooreigel and Khoramshahr populations respectively); ge1-3 – *L. gebelia* (Shafabaksh, Nashoor and Sardasht populations respectively); g – *L. garcinii*; l – *L. laricus*; a – *L. angustissimus*; s1 and 2 – *L. schimperi* (Hasanlangei and Jalaei populations respectively).

The second section is composed of *L. halophilus* (x = 7, SHEIDAI and JALILIAN 2006), third section is composed of *L. laricus* and *L. schimperi* (x = 7) while the forth section is composed of *L. garcinii* (x = 7). Therefore it seems that x = 7, is the dominant basic chromosome number in the species growing in Iran.

A higher coefficient of variation (CV) indicates a higher variation in the size of the chromosomes in the species studied. The highest value of CV among species that have the 2n = 12 chromosome number occurred in the Ghoorigel population of *L. glaber,* and in species with the 2n = 14 chromosome number in the Sardasht population of *L. gebelia*.

Differences in the karyotypic formulae of the species studied and also in distinct populations of a single species indicate the occurrence of structural changes in their chromosomes (inversions, deletions, etc.), which is also supported by species difference in symmetry classes. For example three populations of *L. gebelia* occupy 3 different classes of 1A, 1B and 2B indicating the occurrence of structural changes in their chromosomes. Moreover a significant difference in total chromatin length and the length of short arms ($p < 0.01$) among these populations indicate a significant change in the amount of chromatin (DNA) in them. A similar situation obtains for populations of *L. corniculatus,* as they occupy the 1A and 1B classes of the Stebbins system and differ significantly in their total chromatin length.

Our previous study (SHEIDAI and JALILIAN 2006) also showed that populations of *L. corniculatus* and *L. gebelia* differ significantly in the frequency and position of chiasmata as well as in the number of ring – as well as rod bivalents (which are controlled genetically, COUCOLI et al. 1975, QUICK 1992), indicating a genomic difference among them. Such karyotypic and meiotic variations may be considered a means for local adaptations.

In both clustering and PCO ordination, *L. glaber* shows similarity to *L. corniculatus* and *L. angustissimus* shows similarity to *L. gebelia*, supporting the taxonomic treatment of CHERTKOVA-ZERTOVA (1982), who has placed these species in a single sect. *Lotus*. Our numerical analysis of morphological characters also supports such a grouping (unpublished data).

L. laricus and *L. schimperi* have been placed in the third section *Erythrolotus* (CHERTKOVA-ZERTOVA 1982), but these species are placed in separate clusters/ groups far from each other. A similar result has been obtained from morphological analysis (unpublished data). Based on morphology, *L. garcinii* has been placed in the forth section *Ononidium*. In clustering and PCO ordination it shows some similarity to *L. gebelia*, also supported by numerical taxonomy analysis (unpublished data). Therefore the present karyotypic study only partly supports the taxonomic treatment of CHERTKOVA-ZERTOVA (1982).

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