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Caryology of some iranian species and populations of *Lotus* L.

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Abstract — Cytology of 12 populations belonging to 8 Iranian *Lotus* species was performed revealing the chromosome number, ploidy level, chiasma frequency and distribution, chromosome pairing and segregation for the first time. The chromosome number of 4 species of *L. garcinii*, *L. laricus*, *L. micauxianus* and *L. schimperi* are new reports. The study showed x = 6 and 7 are available in Iranian *Lotus* species, all taxa studied except *L. corniculatus* (4x) were diploid showing bivalent formation in metaphase of meiosis I. A low number of quadrivalents occurred in *L. garcinii* possibly due to hetrozygote translocation.

Key words: Caryology, Iran, *Lotus*

INTRODUCTION

The genus *Lotus* L. of tribe Loteae (Leguminosae) contains a heterogenous assemblage of annual and perennial species distributed widely throughout the world and is comprised of about 100 species (Polhill 1981; 1994a; 1994b). These species are mainly distributed in Mediterranean and NW of America (Polhill and Raven 1981).

The number of *Lotus* species growing in Iran varies according to different authors 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. The genus Lotus is comprised of important forage plants distributed in many regions 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), no such studies exist from Iran. Therefore the present study was performed as a part of biosystematic study of the genus *Lotus* in Iran, reporting chromosome pairing and chiasma frequency of 12 populations of 8 species for the first time trying to illustrate the role of cytological changes in the species diversification.

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MATERIALS AND METHOD

Plant material - Cytological studies were performed in 12 populations of 8 *Lotus* species (Table 1) namely**:** 1- *L. corniculatus* L. (three population), 2- *L. garcinii* DC, 3- *L. gebelia* Vent. (three population), 4- *L. halophilus* Boiss., 5- *L. laricus* Rech. f., 6- *L. michauxianus* Ser., 7- *L. schimperi* Steud. and 8- *L. tenuis* Waldst. & Serg. (Table 1). The voucher specimens are deposited in the herbarium of Shahid Beheshti University (HSBU) and TARI.

Cytological studies - For cytological studies young flower buds were collected randomly from at least 10 randomly selected plants of each species. Flower buds were fixed in ethanol: acetic acid (3:1) for 24 hrs. then washed thoroughly and preserved in ethanol until used The chromosome number, chromosome pairing, chaisma frequency and distribution was determined in minimum 50 metaphase or diakinesis cells while, chromosome segregation was studied in minimum 100 anaphase-I and II stages (Sheidai et al. 1999).

Pollen stainability as a measure of fertility was determined by staining minimum 500 pollen grains with 2 % acetocarmine: 50 % glycerin (1:1) for about ½ hr. Round/ complete pollens which were stained were taken as fertile, while incomplete/ shrunken pollens with no stain were considered as infertile (Sheidai *et al*. 2002).

Analysis of variance (ANOVA) followed by the least significant difference test (LSD) was performed on cytogenetic characteristics including chromosome pairing, chiasma frequency as well as

Table 1 — Meiotic characteristics in *Lotus* species. (Cor = *L. corniculatus* Saheh, Firoozkooh and Nashoor populations respectively, gar = *L. garcinii* Bandar Abbas population, geb = *L. gebelia* Abidar, Naran and Nashoor populations respectively, halo = *L. halophilus* Bandar Abbas population, laric = *L. laricus* Bandar Abbas population, micha = *L. michauxianus* Karaj population, schim = *L. schimperi* Bandar Abbas population, tenu = *L. tenuis* population.

Sp	N	TX	IX	TOX	RB	ROD	TXN	IXN	TOXN	RBN	RODN
cor1	12	16.11	0.80	16.91	4.57	7.42	0.07	1.41	1.34	0.38	0.62
cor2	12	18.26	0.66	18.92	7.00	5.00	0.06	1.58	1.52	0.58	0.42
cor3	12	16.30	1.00	17.30	5.10	6.90	0.08	1.44	1.36	0.43	0.58
gar	7	7.73	0.15	7.88	1.57	4.65	0.02	1.13	1.10	0.22	0.66
geb1	7	10.00	0.42	10.42	3.50	3.50	0.06	1.49	1.43	0.50	0.50
geb2	7	7.68	0.12	7.80	0.76	6.24	0.02	1.11	1.10	0.11	0.89
geb3	7	8.79	0.45	9.24	2.45	4.45	0.06	1.32	1.26	0.35	0.64
halo	7	9.30	1.30	10.60	3.80	3.00	0.19	1.51	1.33	0.54	0.43
laric	7	9.86	1.27	11.13	4.30	2.56	0.18	1.59	1.41	0.61	0.37
micha	7	8.83	0.66	9.49	2.58	4.33	0.09	1.36	1.26	0.37	0.62
schim	7	10.00	0.00	10.00	3.00	4.00	0.00	1.43	1.43	0.43	0.57
tenu	6	9.00	0.65	9.65	3.10	2.90	0.11	1.61	1.50	0.52	0.48

Abbreviations: N = Haploid chromosome number, TX = Terminal chiasmata, IX = Intercalary Chiasmata, TOX = Total chiasmata, RB = Ring bivalent, ROD = Rod bivalent, TXN = Terminal chiasmata/ Chromosome, IXN = Intercalary Chiasmata/ Chromosome, TOXN = Total chiasmata/ Chromosome, RBN = Ring bivalent/ Cell, RODN = Rod bivalent/ Cell.

distribution to indicate any significant difference among the cultivars studied (Sheidai *et al*. 2002). The Statistical analyses were performed with SPSS Ver. 9.

RESULTS

Chromosome number and ploidy level - The results of meiotic analyses are presented in Table 1 and Figures. 1 & 2. The species of *L. tenuis* possessed n = 6 (2n = 2x = 12), *L. garcinii*, *L. gebelia*, *L. halophilus*, *L. laricus*, *L. micauxianus* and *L. schimperi* possessed n = 7 (2n = 2x = 14) and *L. corniculatus* possessed n = 12 (2n = 4x = 24) chromosome number.

The chromosome number of the species *L. corniculatus*, *L. gebelia*, *L. halophilus* and *L. tenuis* reported here supports the earlier studies (Grant 1995; Lövkvist and Hultgård 1999; Manson and Blaise 1995; Snogerup 1985; Vioque and Pastor 1991; Zandstra and Grant 1968), while the chromosome number of *L. garcinii*, L. *laricus*, *L. micauxianus* and *L. schimperi* are new.

Chromosome pairing, chiasma frequency and distribution - All the populations of *L. corniculatus* possessed n = 12 with Firoozkooh population having the highest terminal and total chiasmata (18.26 and 18.93 respectively). The lowest value for the same parameters were observed in Sahneh population (16.11 and 16.92 respectively). The highest value of intercalary chiasmata was observed in Nashoor population (1.00) while, the lowest value for the same occurred in Firoozkooh population (0.66, Table 1).

The tree populations of *L. corniculatus* studied differed in the number of ring as well as rod bivalents (Table 1). The highest number of ring bivalents occurred in Firoozkooh population (7.00) while the lowest value occurred in Sahneh population (4.57).

Three populations of *L. gebelia* possessed n = 2x = 7 chromosome number with Abidar population having the highest values of total and terminal chiasmata (10.50 & 10.07 respectively) and Naran population having the lowest values for the same (7.80 & 7.68 respectively, Table 1). The highest value of intercalary chiasmata occurred in Nashoor population (0.45) and the lowest value occurred in Naran population (0.12). These populations differed significantly in their chiasmata values.

Three populations of *L. gebelia* showed a significant variation in their number of ring and rod bivalents with Abidar population having the highest value of ring bivalents (3.50) and Naran population having the lowest value (0.76). A low value of univalents occurred in Nashoor population (0.08, Table 1).

Among the species having n = 7 chromosome number *L. laricus* possessed the highest values of total and terminal chiasmata (11.04 & 7.69 respectively) while the lowest values occurred in *L. garcinii* (7.69 & 7.73 respectively). The highest value of intercalary chiasmata occurred in *L. halophilus* (1.30) while the lowest value occurred in Naran population of *L. gebelia* (0.12). The species having n = 7 chromosome number formed almost biva-

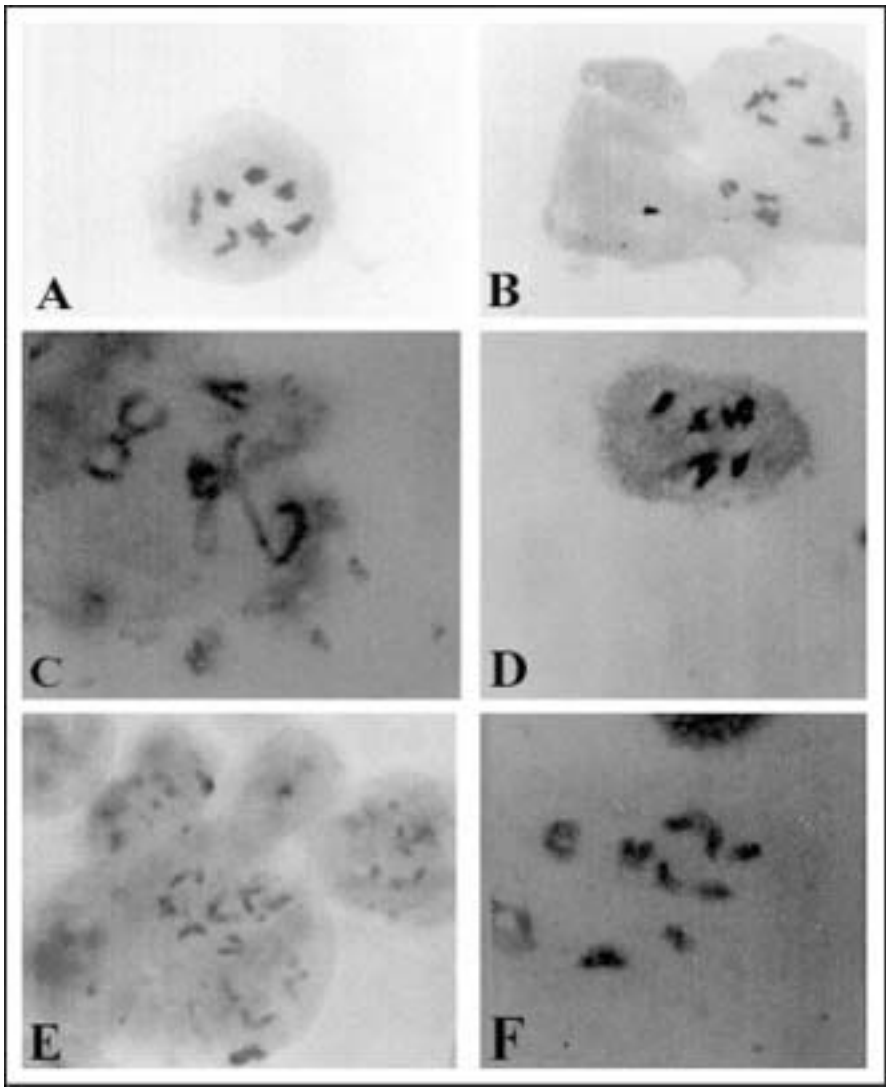

Fig. 1 — Representative metaphase cells in *Lotus* species studied. A = *L. laricus* (n = 7), B = *L. micauxianus* (n = 7), C = *L. garcinii* (n = 7, quadrivalent formed at the left side of the figure), D = *L. schimperi* (n = 7), E = *L. gebelia* (n = 7), F = *L. garcini* (n = 7).

lents in metaphase except *L. garcinii* which formed low value of quadrivalents (0.07, Figure 1).

The highest value of ring bivalents occurred in *L. laricus* (4.30) while the lowest value occurred in Naran population of *L. gebelia* (0.76) followed by *L. schimperi* (3.00). The very low value of ring bivalents observed in *L. gebelia* may be due to early terminalization as these chromosomes move normally during anaphase-I to the cell poles.

Chromosome segregation - Data with regard to chromosome segregation, cytomixis and pollen fertility is provided in Table 2. Anaphase-I laggard chromosomes were observed (Figure 2) in species of *L. corniculatus* (Firoozkooh population = 4.80 %, Nashoor population = 2.00 %) and *L. halophilus* (3.30 %).

Anaphase-II laggards were observed only in Firoozkooh population of *L. corniculatus* (1.9 %). The chromosome stickiness occurred in both anaphase-I and II almost in al the species studied (ranging from 2.00-25.20 %) except in *L. schimperi* and *L. tenuis*.

Another interesting cytogenetic abnormality observed was the occurrence of cytomixis and chromatin migration in all the species studied except *L. halophilus* (Figure 2). Chromatin migration occurred among the neighboring meiocytes from early prophase to late anaphase-I (Figure 2).

The *Lotus* species studied showed in general a high pollen fertility ranging from 90-99 %.

Table 2 — Chromosome segregation, cytomixis and pollen fertility in *Lotus* species studied. (The name of species as in Table 1.).

SP	A1L	A2L	A1S	A2S	CYTO	PF
cor1	0.00	0.00	1.00	0.00	23.10	98.00
cor2	4.80	1.90	25.20	9.90	25.00	86.00
cor3	2.00	0.00	7.00	0.00	0.00	99.60
gar	0.00	0.00	0.00	0.00	19.52	98.00
geb1	0.00	0.00	4.00	3.40	23.00	99.50
geb2	0.00	0.00	5.00	6.00	19.00	99.00
geb3	0.00	0.00	2.00	3.00	16.00	99.00
halo	3.30	0.00	7.00	0.00	0.00	96.00
laric	0.00	0.00	0.00	0.00	10.00	99.00
mich	0.00	0.00	10.00	8.00	30.00	90.00
schim	0.00	0.00	0.00	0.00	45.00	99.00
tenu	0.00	0.00	0.00	0.00	20.00	98.00

Abbreviations: A1L = Laggards in anaphase-I, A2L = Anaphase-II laggards, A1S = Anaphase-I stickiness, A2S = Anaphase-II stickiness, CYTO = Cytomixis, PF = Pollen fertility, All values in %.

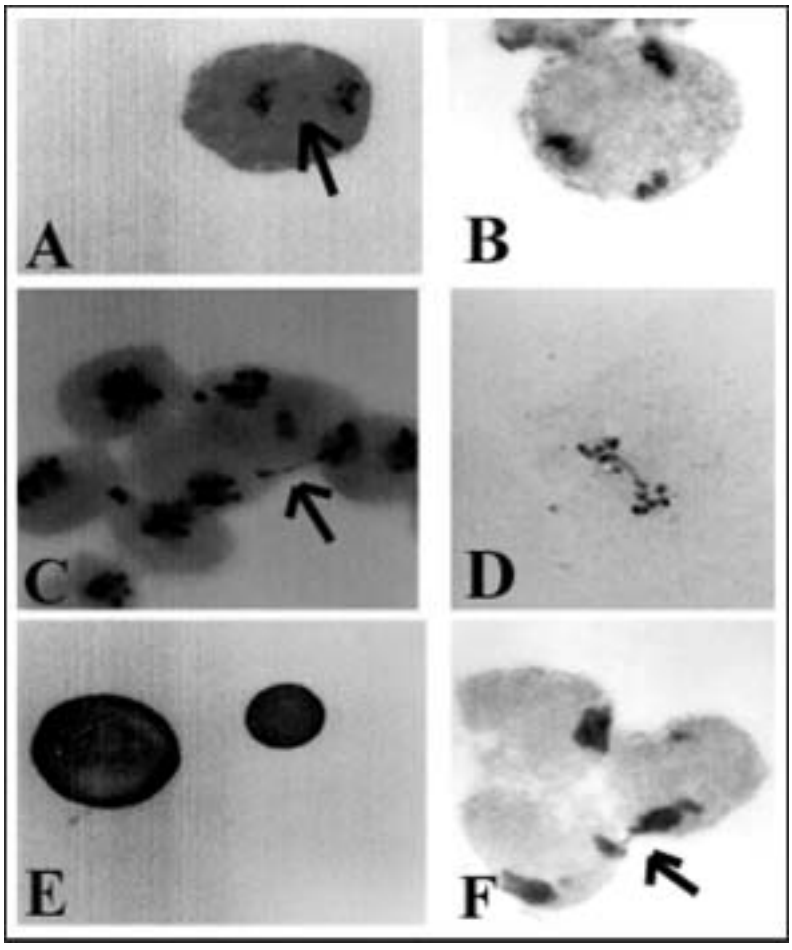

Fig. 2 — Representative meiotic cells showing chromosome segregation, cytomixis and pollen fertility in *Lotus* species studied. A & B = Anaphase-I cells showing laggard chromosome in *L. corniculatus*, C = Cytomixis in *L. garcinii*, D = Stickiness in *L. halophilus*, E = Fertile (larger pollen) and infertile pollen (smaller pollen) in *L. schimperi*, F = Cytomixis in *L. schimperi*.

DISCUSSION

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-chomosomed 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). CHERTKOVA-ZERTOVA (1982) taxonomic treatment of the genus is the most recent and complete one. He 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 comprised of *L. tenuis*, *L. krylovii*, *L. corniculatus*, *L. angustissimus* (all having x = 6), *L. gebelia* and *L. micauxianus* (possessing x = 7).

The second section is comprised of *L. halophilus* (x = 7), third section is comprised of *L. laricus* and *L. schimperi* (x = 7) while the forth section is comprised of *L. garcinii* (x = 7).

Although *L. corniculatus* is a tetraploid species all populations studied formed only bivalents and univalents with no quadrivalent formation. DAWSON (1941) reported mostly the occurrence of bivalents in meiosis of *L. corniculatus* along with tetrasomic inheritance, which led STEBBINS to consider this species as a segmental allopolyploid (GRANT 1995). Merely bivalent formation of *L. corniculatus* populations of Iran may further support such consideration.

L. garcinii which is diploid (n = 7) formed low value of quadrivalents possibly due to the occurrence of a heterozygote translocation between two non-homologous chromosomes leading to the formation of gametes with new genetic linkage. Such a genomic change may be used for local adaptations as also reported in other diploid species such as *Aegilops* (SHEIDAI *et al*. 2002).

ANOVA test performed on cytogenetic characteristics among the 3 populations of *L. corniculatus* and among the species having n = 7 showed presence of a significant difference almost among all the species and populations indicating their genomic differences possibly occurred during *Lotus* species diversification. Variation in chiasma frequency and localization is genetically controlled (QUICK 1992) and has been reported in several plant species as well as in crop plant varieties (REES and JONES 1977). Such a variation in the species/ populations with the same chromosome number is considered as a means for generating new forms of recombination influencing the variability within natural populations in an adaptive way (COUCOLI *et al*. 1975; REES and DALE 1974).

In order to check if any specific control exists over chiasma frequency and distribution in the species having n = 6, 7 and 12, relative chiasmata data (chiasmata number divided by haploid chromosome number) was used which is not affected by the chromosome number and comparison of species with different chromosome numbers may be performed (SHEIDAI *et al*. 2002). The comparison of relative chiasmata data showed no specific values for species with similar haploid chromosome number. For example the relative chiasmata values of the species having n = 6 is close to species having n = 7 as well as 12 and no distinction exists among them (Table 1). Moreover correlation test performed between chromosome number and relative chiasmata values in *Lotus* species did not show a significant correlation i.e. with increase in chromosome number no significant increase in relative chiasmata values occurs.

In some plant groups such as *Stipa* (Poaceae) our earlier studies showed a decrease in relative chiasmata values with increase in ploidy level and that each ploidy group i.e. diploid, tetraploid and hexaploid species possesses specific distinct relative chiasmata values (SHEIDAI *et al*. 2003). The higher values of relative chiasmata in the species having lower chromosome number is suggested to be related to more genetic recombination required by these plants (SHEIDAI *et al*. 2003).

Cytomixis and migration of chromatin material among the adjacent meiocytes occurs through cytoplasmic connections originated from the pre-existing systems of plasmodesmata formed within the anther tissues. The plasmodesmata become completely obstructed by the deposition of callose (SHEIDAI 1997), but in some cases they persist during meiosis and increase in size forming conspicuous inter-meiocytes connections or cytomictic channels that permit the transfer of chromosomes. Cytomixis leads usually to aneuploidy and reduction in fertility of plants, therefore it is considered to be of less evolutionary significance. However it may bring about new genetic variability by producing aneuploid gametes and new phenotypic characters as reported in other plants (SHEIDAI *et al*. 1993).

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