KARYOTYPE AND SEED PROTEIN ANALYSES OF *LYCIUM* (*SOLANACEAE*) IN IRAN

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Twelve populations of six *Lycium* species were analysed for karyotypic characters and seed storage proteins using multivariate statistical methods. All reported chromosome numbers (mostly new) are $2n = 24$ ($x = 12$). Karyotypes were symmetrical and placed in 1A and 2A classes of Stebbins' karyotype classification. Cluster analysis of karyological and protein data revealed variations among the populations of *L. depressum* and *L. ruthenicum*, and supports close relationships of *L. kopetdaghi* and *L. depressum* with *L. makranicum*, and *L. shawii* with *L. edgeworthii*.

Keywords. Cluster analysis, symmetrical karyotype.

INTRODUCTION

The genus *Lycium* comprises about 100 woody species (D'Arcy, 1979) that grow mainly in arid or semi-arid environments. It originated in South America (Bernardello, 1987), and is included in subfamily *Solanoidea*, which is considered to be monophyletic and derived (Olmstead & Palmer, 1992). Seven endemic *Lycium* species and subspecies have been reported from temperate regions of Iran (Khatamsaz, 1998).

Although there are some reports on karyotypic features of *Lycium* species from other countries (Stiefkens & Bernardello, 1996), in Iran chromosome numbers have been reported for two species only (Lessani & Chariat-Panahi, 1979; Ghaffari, 1987). Moreover, to our knowledge, seed proteins have not previously been used in the biosystematics of *Lycium*. Seed protein studies in *Solanum* (Edmonds & Glidewell, 1977) revealed the value of protein data in taxonomy of the genus and provided evidence for interpopulation divergence. The present paper uses both karyotypic and seed protein data to clarify the species relationships, and seeks to elucidate chromosomal changes occurring during speciation.

MATERIALS AND METHODS

Plant material

Seeds of six *Lycium* species were collected from 12 natural populations for protein analyses and karyological studies (Table 1). Voucher specimens are deposited in the central herbarium of Iran (TARI).

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TABLE 1. Species/populations, their locality and voucher number

Species/population	Locality	Voucher number
Lycium ruthenicum Murray	Khorasan	8557
L. ruthenicum	Semnan	1192
L. ruthenicum	Azarbayejan	73148
L. shawii Roemer & Schultes	Hormozgan	45122
L. shawii	Balochestan	43232
L. depressum subsp. *depressum* Schonebeck-Temesy	Tehran	16002
L. depressum	Fars	61784
L. depressum	Khorasan	18908
L. depressum	Semnan	40959
L. kopetdaghi Pojark	Kopetdagh	35669
L. makranicum Schonebeck-Temesy	Balochestan	53271
L. edgeworthii Dun.	Boshehr	67494

Cytological preparation and karyotypic analyses

Seeds were germinated on petri-plates and fresh root-tips were collected for karyological studies. Experimentation with different pretreatments showed that the best results were obtained at 18°C from 8-hydroxyquinoline (2h). Other steps in cytological preparations followed the methods of Sheidai *et al.* (1996a), using 2% acetic orcein. At least 10 well-spread metaphase plates sketched by camera lucida were used for karyotypic analyses. Chromosomes/karyotypes were compared following the criteria of Levan *et al.* (1964). In order to detect significant differences in the size of chromosomes among the species/populations, factorial analyses (ANOVA) was performed using species and chromosomes as the two factors, followed by the least significant difference test (LSD) (Sheidai *et al.*, 1996b).

In order to group the species/populations having karyological similarity, cluster analyses using single linkage and WARD methods was performed (Bernardello *et al.*, 1994; Sheidai *et al.*, 1996b; Sheidai & Inamdar, 1997). For cluster analyses variables were standardized (mean = 0, variance = 1; Chatfield & Collins, 1995).

In order to determine the most variable chromosome/chromosome arms among the species, factor analyses based on principal components analyses (PCA) was performed on standardized karyological data. Varimax rotation was carried out after Kaiser normalization (Manly, 1986). Ordination of the species was performed on the first two principal components axes (McClintock & Waterway, 1994; Sheidai *et al.*, 1996c; Sheidai & Alishah, 1998).

Protein extraction, electrophoresis and data analyses

One hundred mg of each sample (25–50 seeds) was homogenized to obtain a fine powder. Proteins were extracted in precooled mortar and pestle over ice with a

0.39M Tris phosphate buffer (pH 8.3). The resulting mixture was centrifuged at 15,000g for 10min. The crude extracts were boiled for 5min in 77mM Tris-HCl (pH 6.8), 4% sodium dodecyl sulphate (SDS), 10% 2-mercaptoethanol and 3% glycerol (Sanches-Yelamo *et al.*, 1995). Protein electrophoresis by SDS–PAGE used 20mg of protein in each lane. Vertical slab gels 1mm thick were electrophoresed at a constant current of 30mA for 8h. Coomassie Briliant Blue G-250 was used for overnight gel staining followed by trichloroacetic acid as fixative.

To estimate species/population similarity as indicated by protein electrophoresis patterns, simple matching coefficient (SM) and Jaccards' indices were determined (Digby & Kempton, 1994). Each protein band was considered as a qualitative character and coded as 1 (presence) versus 0 (absence) (Carrens *et al.*, 1997). The resulting data matrix was used for cluster analyses using single linkage method. In order to identify the most variable protein bands among the species/populations studied, principal components analyses (PCA) was performed. Statistical analyses used SPSS (Norusis, 1988) and NTSYS ver. 1.4 (Rohlf, 1987) software.

RESULTS AND DISCUSSION

The somatic chromosome number and details of karyotypes of each species/population are presented in Table 2. In all the taxa studied $2n=24$ was observed (Figs 1, 2). The chromosome numbers found in *L. ruthenicum* and *L. depressum* support the previous reports (Lessani & Chariat-Panahi, 1979; Ghaffari, 1987), whereas somatic chromosome numbers of *L. shawii*, *L. kopetdaghi* and *L. makranicum* are reported for the first time. Karyotypic study of South American *Lycium* has also shown the presence of $2n=24$ (Stiefkens & Bernardello, 1996). Considering $x=12$ as the basic chromosome number of the genus, all the species/populations studied are diploid.

The highest value for total chromatin length, size of the longest chromosome and size of the shortest chromosome was observed in *L. ruthenicum* (Semnan: 70.87, 9.00 and 4.25μm respectively) and the lowest value in *L. shawii* (40.92, 4.70 and 2.52μm respectively) (Table 2). Stiefkens and Bernardello (1996) reported 21.52–28.01μm as the range of total haploid chromatin length in South American *Lycium* which differs from 40.92–70.87μm occurring in the present study. Adaptations of the species to different environmental conditions/difference in nuclear DNA content might be the reason for such differences.

Factorial analyses (ANOVA) for size of the chromosomes showed significant differences ($P<0.01$) for both factors of the chromosomes and species/populations, indicating that at least two species/populations differ in the size of chromosomes. Pair-wise LSD tests showed no significant differences among the populations of *L. ruthenicum* and *L. depressum*, indicating interpopulation uniformity in total chromatin length, as suggested for South American *Lycium* (Stiefkens & Bernardello, 1996); however, LSD tests showed significant differences among the species studied, indicating that speciation might have been accompanied by loss or gain in chromatin material.

TABLE 2. Karyotypic details of Iranian *Lycium*

Lycium population	Karyotype formulae	Stebbins' class	TL (μm)	L (μm)	S (μm)	r	SAT no.	SAT-Chr
L. ruthenicum (Semnan)	9m + 3sm	2A	70.87	9.00	4.25	1.50	1	1
L. ruthenicum (Khorasan)	9m + 3sm	2A	66.84	7.87	3.87	1.52		
L. shawii	11m + 1sm	2A	40.92	4.70	2.24	1.35	1	4
L. depressum (Tehran)	11m + 1sm	2A	56.48	6.25	3.59	1.44	2	1, 10
L. depressum (Fars)	12m	1A	64.08	7.27	3.72	1.30	1	5
L. depressum (Khorasan)	12m	1A	67.18	7.65	4.05	1.83	1	6
L. kopetdaghi	12m	1A	50.91	5.71	3.09	1.38	1	7
L. makranicum	12m	1A	51.36	5.97	3.17	1.32	2	8, 12

Abbreviations: SAT no., number of SAT-chromosomes; SAT-Ch, SAT-chromosome; TL, total chromatin length; L, longest chromosome; S, shortest chromosome; r, mean arm ratio.

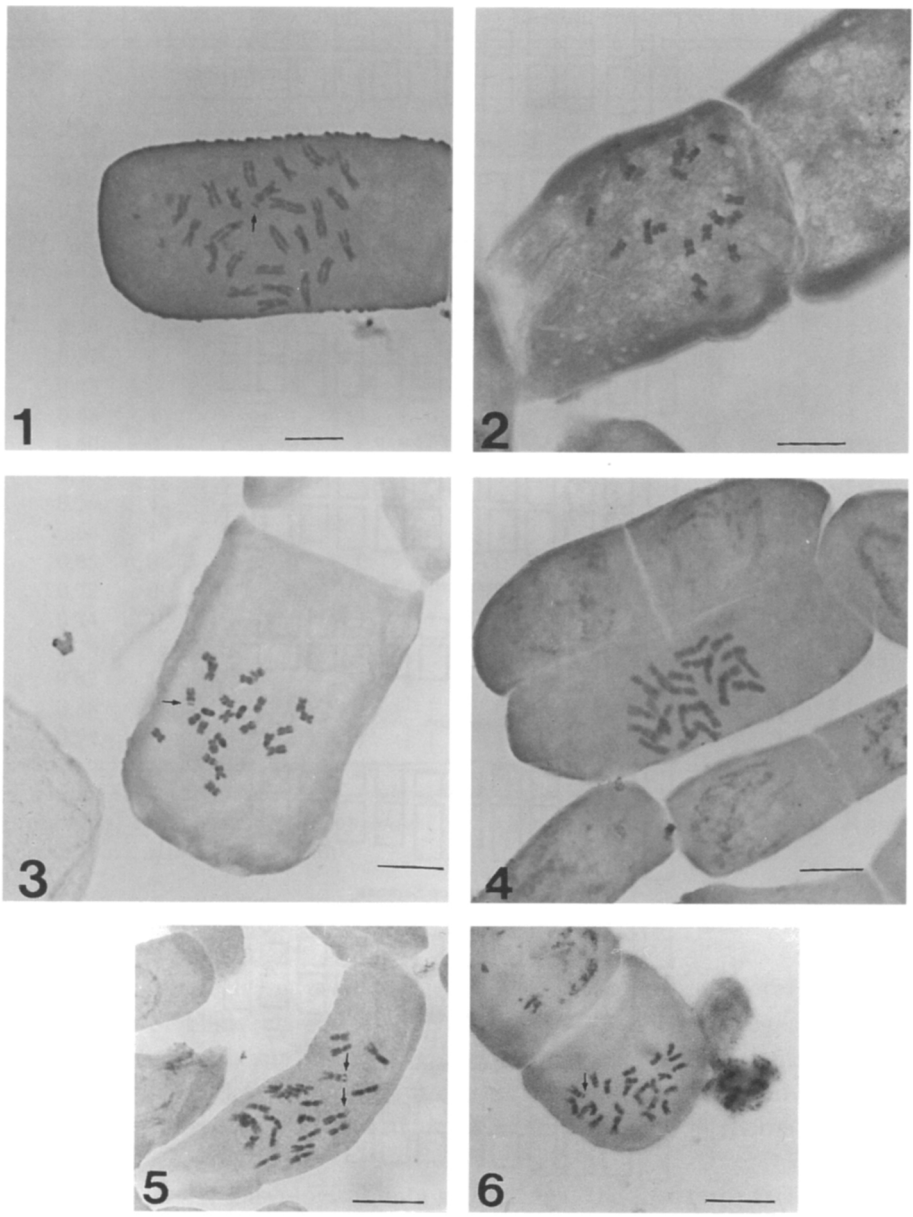

FIG. 1. Representative somatic chromosomes in Lycium. 1, *Lycium depressum* (Khorasan); 2, *L. makranicum*; 3, *L. shawii*; 4, *L. kopetdaghi*; 5, *L. depressum* (Fars), and 6, *L. depressum* (Tehran). Arrow indicates the satellite; scale bar = 10μm.

The number of SAT-chromosomes is presented in Table 2. Terminal satellites were located at the distal end of short arms as well as the long arms (Table 2, Fig. 2). American *Lycium* show generally uniform karyotypes (Stiefkens & Bernardello,

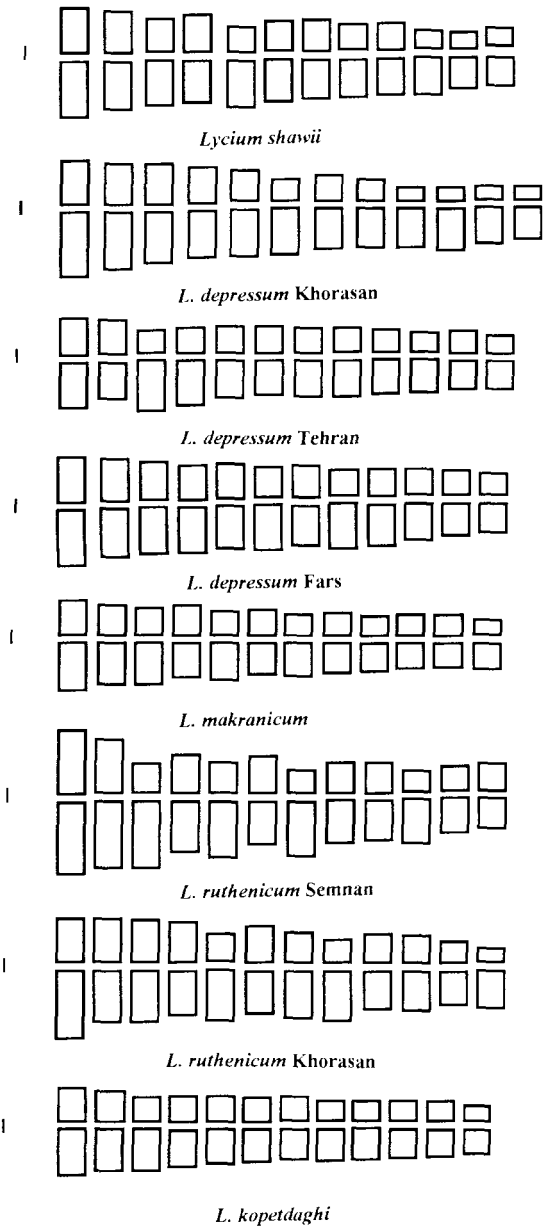

FIG. 2. Idiograms of *Lycium*. Vertical bar = 1 μm.

1996), microsatellites are present in chromosome pair 1 and are attached to the short arm. In Iranian taxa studied, there is variation in the chromosome on which the satellite is located, and in some species two satellite chromosomes are present. Moreover microsatellites are attached to the short as well as long arm indicating a

TABLE 3. Distribution of protein bands across 12 populations, plus RM values

		Species/population											
Band	RM	1	2	3	4	5	6	7	8	9	10	11	12
1	0.05	0	0	0	0	0	1	0	0	1	1	1	0
2	0.11	0	0	0	0	0	1	0	0	1	1	1	0
3	0.20	1	1	0	0	0	1	0	0	1	1	1	1
4	0.27	0	0	0	0	0	1	1	1	1	1	1	0
5	0.31	1	1	1	1	1	1	0	0	1	1	1	1
6	0.34	1	1	1	1	1	1	1	1	1	1	1	1
7	0.37	1	1	1	1	1	1	1	0	1	1	1	1
8	0.43	0	0	1	0	0	0	0	0	1	1	1	1
9	0.48	1	1	1	1	0	0	0	0	0	1	1	1
10	0.50	0	1	0	0	0	0	0	1	1	1	1	1
11	0.53	1	1	1	1	1	1	1	1	1	1	1	1
12	0.56	1	1	1	1	1	1	1	1	1	1	1	1
13	0.64	1	1	1	1	0	1	0	1	1	1	1	1
14	0.65	0	0	0	0	0	0	0	0	1	1	1	1
15	0.72	0	0	0	0	1	1	0	1	0	0	1	0
16	0.74	1	1	1	1	1	1	1	1	1	1	1	1
17	0.78	1	1	1	1	1	1	1	1	1	1	1	1
18	0.82	1	1	1	1	1	1	1	1	1	1	1	1
19	0.88	1	1	1	1	1	0	0	1	0	1	1	1
20	0.94	1	1	1	1	1	1	1	1	1	1	1	1

Populations 1–12 respectively are: *L. ruthenicum* (Azarbayejan, Semnan, Khozestan), *L. shawii* (Hormozgan, Balochestan), *L. copetdaghi*, *L. makranicum*, *L. edgeworthii*, *L. depressum* (Semnan, Fars, Khorasan, Tehran).

greater degree of karyotype repatterning occurring among Iranian taxa compared with South American ones.

Karyotype asymmetry is presented in Table 2. Taxa studied conformed to class 1A and 2A in Stebbins' classification (Stebbins, 1971) which are regarded as primitive and symmetrical, supporting the results obtained in South America *Lycium* (Stiefkens & Bernardello, 1996). Symmetrical karyotypes have been reported for other *Solanaceae* species too (Bernardello & Anderson, 1990; Bernardello *et al.*, 1994).

Factor analyses of karyological data showed that the first three factors comprise about 86% of total variance. The most variable characters in the first factor are: The total length of chromosomes 1–12, long arm of chromosomes 1, 2, 4, 6, 8–11 and short arm of 2, 5, 8 and 11. In the second factor, S/L of chromosomes 6 and in the third factor, S/L of chromosomes 3 and 7 possess the highest positive correlation with the factor. Stiefkens and Bernardello (1996) showed that no major chromosomal rearrangements have occurred in *Lycium* species, an interpretation also supported by the present findings; change in arm ratio is confined to 3 chromosomes only.

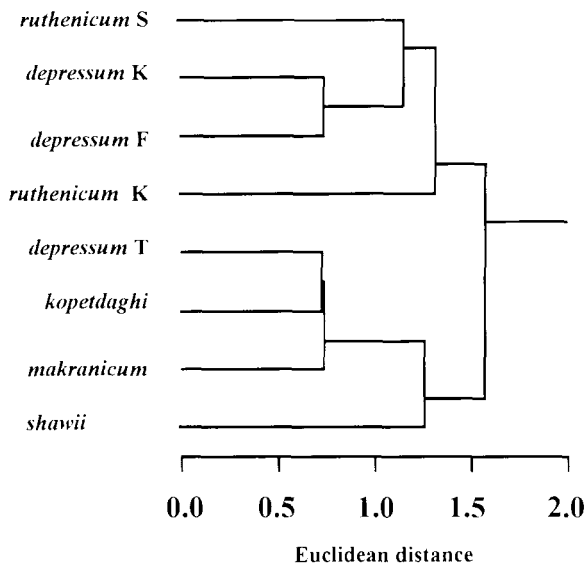

FIG. 3. Cluster analysis (single linkage) of *Lycium* karyotypic data. Abbreviations as Fig. 5.

The bands obtained from SDS-analyses and RM values are presented in Table 3. Bands 11, 12, 16, 18 and 20 are present in all sampled populations. Bands 8 and 10 are present only in *L. depressum*, and hence might be taken as species specific. Bands 8 and 10 are present in *L. depressum* and *L. ruthenicum*, though *L. edgeworthii* also possesses band 10. Two populations of *L. shawii* differ only in three bands (4, 13, 15). Principal components analyses of the protein bands indicated that the first three components comprise about 77% of the total variance. Bands 1, 3–6 and 8–11 possess the highest correlation with components 1 and 2, being the most variable bands among the taxa studied, suggesting that the genes coding these proteins have undergone changes during speciation.

Grouping of the species/populations based on karyotypic and protein similarities are presented in Figs 3–5. Cluster analysis and PCA ordination produced the same results. In karyotypic analyses (Fig. 3), two clusters are formed; the first one is comprised of *L. depressum* (Fars and Khorasan populations) and *L. ruthenicum* (Semnan and Khorasan populations). The Khorasan population of *L. ruthenicum* shows some differences from the Semnan population (Figs 3, 4), a pattern repeated in the cluster analyses of protein data (Figs 5–7). This is true also for Tehran population of *L. depressum* showing some differences from Khorasan and Fars populations. The second cluster is comprised of *L. depressum* Tehran, *L. kopetdaghi*, *L. makranicum* and *L. shawii*.

Cluster analyses of protein data (Fig. 5) produced three clusters; the first cluster is comprised of *L. depressum* (Tehran, Khorasan, Fars, Semnan), *L. ruthenicum* (Semnan, Azarbayejan, Khorasan), *L. edgeworthii* and *L. kopetdaghi*. The second

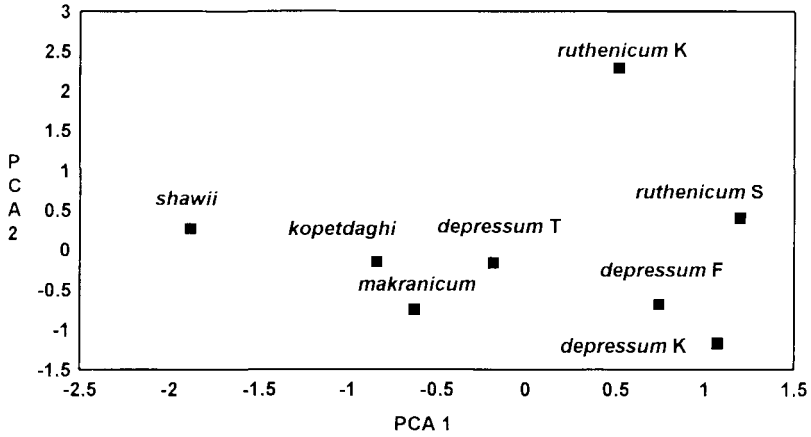

FIG. 4. Ordination of *Lycium* karyotypic data on the first two principal components axes. Abbreviations as Fig. 5.

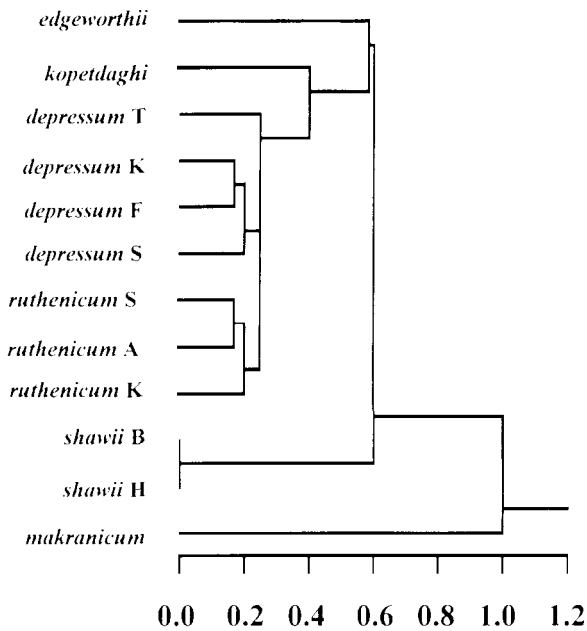

FIG. 5. Cluster analysis (single linkage) of *Lycium* protein data. Abbreviations: A, Azarbayejan; S, Seman; K, Khorasan; H, Hormozgan; B, Balochestan; T, Tehran; F, Fars.

cluster is comprised of *L. shawii* (Balochestan, Hormozgan) and the third cluster is formed by *L. makranicum*.

Different populations of *L. shawii* show similarity to *L. edgeworthii*, and *L. makranicum* to *L. shawii*. Different populations of *L. depressum* are placed close to populations of *L. ruthenicum*, and *L. kopetdaghi* shows similarity with *L. depressum*

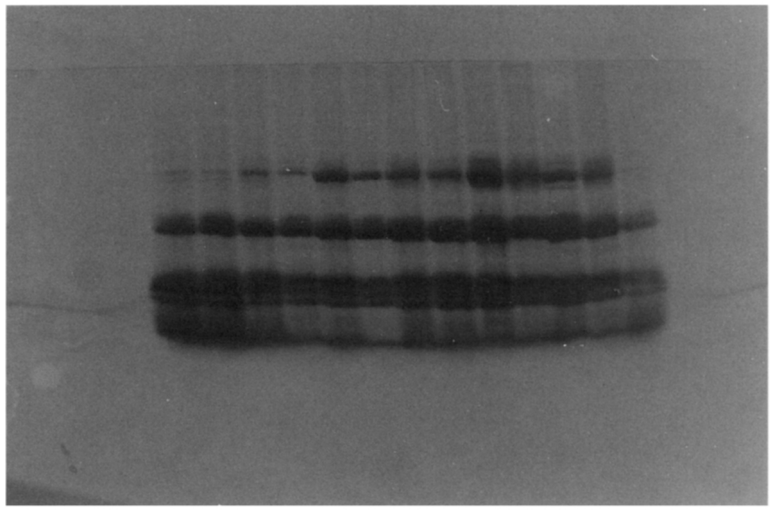

FIG. 6. Electrophrogram (SDS-PAGE) of species/populations studied; sequence of taxa from left to right as Table 3; columns 1 and 2 belong to the first species.

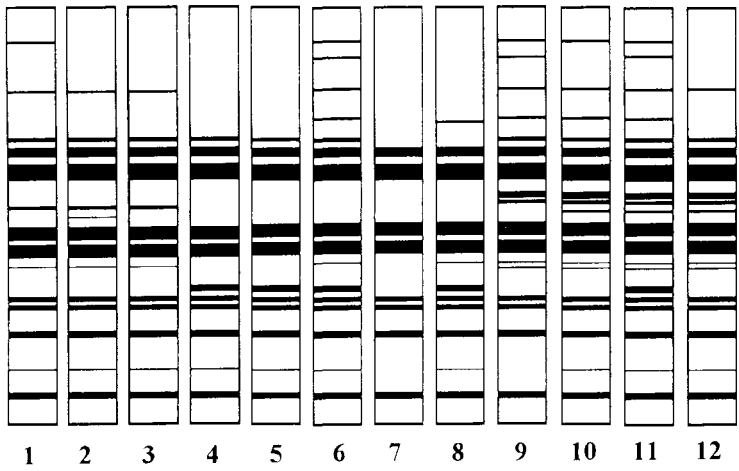

FIG. 7. Diagram showing protein bands. Sequence of species/populations as in Table 3.

Tehran, supporting the cluster analyses of karyological data (except differences observed between *L. depressum* Tehran and the other populations).

Khatamsaz (1998), in her taxonomical treatment of the *Lycium*, considered *L. kopetdaghi*, *L. depressum* and *L. makranicum* to be close to each other, *L. ruthenicum* to be close to *L. depressum*, and *L. shawii* to be close to *L. makranicum* and *L. edgeworthii*. Cluster analyses of karyological and protein data supports Khatamsaz's (1998), taxonomic treatment, providing additional evidence for the species relationships and interpopulation differences in karyological and protein characteristics.

ACKNOWLEDGEMENTS

This research was supported by the Office of Planning, Programming and Coordinating research affairs of The Ministry of Jihad Sazandeghi. The referees are thanked for their helpful and constructive criticisms of our manuscript.

REFERENCES

BERNARDELLO, L. M. (1987). Comparative floral morphology in *Lycieae* (*Solanaceae*). *Brittonia* 39: 11–129.

BERNARDELLO, L. M. & ANDERSON, G. (1990). Karyotypic studies in *Solanum* section *Basarthrum* (*Solanaceae*). *Bol. Acad. Nac. Ci.* 57: 173–356.

BERNARDELLO, L. M., HEISER, C. B. & PIZZANO, M. (1994). Karyotypic studies in *Solanum* section *Lasiocarpa* (Solanaceae). *Amer. J. Bot.* 81: 95–103.

CARRENS, M. E., FUENTES, E. & MERINO, E. F. (1997). Seed protein patterns of nine species of *Cactaceae*. *Biochem. Sys. Eco.* 26: 43–49.

CHATFIELD, C. & COLLINS, A. J. (1995). *Introduction to Multivariate Analysis*. London: Chapman & Hall.

D'ARCY, W. J. (1979). The classification of the Solanaceae. In: HAWKES, J. G., LESTER, R. N. & SCHELDING, A. D. (eds) *The Biology and Taxonomy of the Solanaceae*, pp 3–48. London: Academic Press.

DIGBY, P. G. N. & KEMPTON, R. A. (1994). *Multivariate Analysis of Ecological Communities*. London: Chapman & Hall.

EDMONDS, J. M. & GLIDEWELL, S. M. (1977). Acrylamide gel electrophoresis of seed proteins from some *Solanum* (section *Solanum*) species. *Plant Syst. Evol.* 127: 277–291.

GHAFFARI, S. M. (1987). Chromosome counts of some angiosperms from Iran, II. *Iran. J. Bot.* 3: 183–188.

KHATAMSAZ, M. (1998). *Flora of Iran* (Solanaceae). Tehran: Forage and Range Land Research Institute.

LESSANI, H. & CHARIAT-PANAHI, M. S. (1979). In IOPB chromosome number reports LXV. *Taxon* 28: 635–636.

LEVAN, A. K., FREDGA, K. & SANDBERG, A. A. (1964). Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201–220.

MANLY, B. F. J. (1986). *Multivariate Statistical Methods: a Primer*. London: Chapman & Hall.

McCLINTOCK, K. A. & WATERWAY, M. J. (1994). Genetic differentiation between *Carex lasiocarpa* and *C. pellita* (Cypraceae) in North America. *Amer. J. Bot.* 8: 224–231.

NORUSIS, M. J. (1988). *SPSS/PC Advanced Statistics*. Chicago: SPSS Inc.

OLMSTEAD, R. & PALMER, J. (1992). A chloroplast DNA phylogeny of the Solanaceae: Subfamilial relationships and character evolution. *Ann. Miss. Bot. Gar.* 79: 346–360.

ROHLF, F. J. (1987). NTSYS-PC. *Numerical Taxonomy and Multivariate Analysis System for the IBM-PC Microcomputer (and Compatible), Ver. 1. 4*. Steauket, New York: Applied Biostatistics Inc.

SANCHES-YELAMO, M. D., ESPENJO-IBANEZ, M. C., FRANCISCO-ORTEGA, J. & SANTOSE-GUERRA, A. (1995). Electrophoretical evidence of variation in populations of the fodder legume *Chamaecytisus proliferus* from the Canary Islands. *Bioch. Syst. Ecol.* 23: 53–63.

SHEIDAI, M. & ALISHAH, O. (1998). Morphometric studies in *Gossypium herbaceum* cultivars in Iran national Genebank. *Plant. Genet. Resour. Newsl.* 113: 44–46.
SHEIDAI, M. & INAMDAR, A. C. (1997). Cytomorphology of *Asparagus* taxa using multivariate analyses. *The Nucleus* 40: 7–12.
SHEIDAI, M., AHMADIAN, P. & POORSEYEDY, SH. (1996a). Cytological studies in Iran zira from three genera: *Bunium*, *Carum* and *Cuminum*. *Cytologia* 61: 19–25.
SHEIDAI, M., MASSOUMII, A. R. & PAKRAVAN, M. (1996b). Karyotypes of some *Astaragalus* taxa (sec. *Xiphidium*) from Iran. *The Nucleus* 38: 111–113.
SHEIDAI, M., VOJDANI, P. & ALISHAH, O. (1996c). Karyological studies in *Gossypium herbaceum* cultivars of Iran. *Cytologia* 61: 365–374.
STEBBINS, G. L. (1971). *Chromosomal Evolution in Higher Plants*. London: E. Arnold.
STIEFKENS, L. & BERNARDELLO, L. M. (1996). Karyotypic studies in South American *Lycium* (Solanaceae). *Cytologia* 61: 395–402.

Received 5 November 1998; accepted with revision 1 February 1999