CYTOLOGY, MORPHOMETRY AND SEED PROTEIN ANALYSIS OF SOLANUM SPECIES IN IRAN

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Biosystematic study was performed on several *Solanum* species native to Iran. Cytological analysis revealed presence of 2n = 24, 48 and 72 chromosome number in the species/populations studied. Karyotypic studies revealed the role played by polyploidy and structural changes of chromosomes during species diversification. The somatic chromosome number of *S. persicum* is reported for the first time.

Numerical analysis of morphological characters and SDS-PAGE protein bands supported the classical taxonomic treatment of the genus, providing evidence for the species inter-relationships.

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Key words. Solanum, chromosomes, karyotype, numerical analysis, protein, Iran.

بررسی سیتولوژیکی، مورفومتری و پروتئینی گونه‌های Salanum ایران

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بررسی بیوسیستماتیکی گونه‌های *Solanum* ایران انجام گرفت. مطالعات سیتولوژیکی وجود تعداد کروموزوم‌های سوماتیکی ۲۴، ۴۸ و ۷۲ را در گونه‌ها و جمعیت‌های مطالعه شده نشان داد. بررسی‌های کاریوتیپی نقش پلی‌پلوئیدی و تغییرات ساختمانی کروموزوم‌ها را در فرایند گونه‌زایی مشخص کرد. تعداد کروموزوم گونه *S. persicum* برای اولین بار گزارش می‌شود. تجزیه و تحلیل صفات ریختی و پروتئینی با استفاده از روشهای تاکسونومی عددی، علاوه بر تأیید رده‌بندی تاکسونومیکی گونه‌ها، قرابت‌های میان گونه‌ها را نیز مشخص کرد. باندهای پروتئینی ویژه گونه‌ها مشخص شدند. متغیرترین صفات ریختی و باندهای پروتئینی که در روند گونه‌زایی تغییر کرده‌اند شناسایی شدند.

INTRODUCTION

The genus *Solanum* of *Solanaceae* consists of about 1400 species distributed all over the world and 12 species in Iran. *Solanum* species are of medicinal and economical importance due to the presence of alkaloids such as solanine, solanidine and solasodine (Barbosa-Filho & Agra 1991).

Khatamsaz (1998) in a taxonomic treatment considered two subgenera for the genus *Solanum*: 1- *Solanum* and 2- *Leptostemonum* (Dun.) Bitter. The subgenus *Solanum* is comprised of two sections, *Solanum* and *Dulcamara* (Dun.) Bitter while *Leptostemonum* is comprised of a single section *Melongena* Neess. Although there has been several studies on taxonomy and cytology of *Solanum* species in the other countries (Kirti & Rao 1982, Trivedi & Sinha 1986, Bernardello & al. 1994), there has been no report on cytology, numerical taxonomy and seed protein analysis of *Solanum* species in Iran. The present study considers karyotypic, morphometric and seed protein analysis of *Solanum* species growing in Iran trying to through light on the species inter-relationships and inter-populations divergence.

MATERIALS AND METHODS

Plant materials

The name of the species/populations studied and voucher numbers are presented in Table 1. Three to 5 plants were studied for morphological characters in each population/species. Voucher specimens are deposited in the Central Herbarium of Iran (TARI) and the herbarium of Mashhad University.

Morphometry

Forty quantitative and qualitative morphological characters were studied (Table 2). The qualitative characters were coded as multistate characters while the mean of quantitative characters were used for analysis (Sneath & Sokal 1973). Multivariate statistical analysis used standardized data (mean = 0, variance = 1: Manly 1986).

Grouping of the species/populations of each section was carried out separately by using single linkage clustering method based on the

Table 1. *Solanum* species their locality and voucher number.

Species/population	Locality	Code	Voucher
Subgenus Solanum			
Section Solanum			
Solanum nigrum L.	Azarbayejan, Arasbaran	A	24399 Assadi, Sardabi
S. nigrum	Mazandaran, Nowshahr	M	33808 Assadi
S. nigrum	Tehran, 20 Km to Karaj	T	31892 Buttler
S. nigrum	Khuzestan, Masjed Soleyman	KH	72957 Khatamsaz
S. olgae Pojark.	Tehran, Damavand	T	32530 c
S. olgae	Gorgan, Kalateh	G	12929 Hashemi
S. alatum Moench	Bandar Abbas, Geno mountain	B	39751 Mozaffarian, Hashemian
S. luteum Miller	Tehran, Chitgar	T	1912 Sabeti
S. luteum	Bandar Abbas, Geno mountain	B	52364 Mozaffarian
S. luteum	Esfahan, Fereydoonshahr	E	7442 Golhoni
Section Dulcamara			
S. kieseritzkii C.A.Mey.	Gilan, Asalem	GI	73151 Khatamsaz, Farzaneh
S. persicum Willd.	Gilan, Astara	GI	14253 Izadyar
S. dulcamara L.	Tehran	T	47698 Akhani
S. dulcamara	Fars, Bakhtiari	F	9184 Feyzi
S. asie-mediae Pojark.	Khorasan, Mashhad	K	10536 Mashhad University
Subgenus Leptostemonum			
Section Melongena			
S. incanum L.	Balouchestan, Pasa bandar	BA	52850 Mozaffarian
S. incanum	Bandar Abbas, Geno mountain	B	15536 Wendelbo, Foroughi
S. surattense Burm. f.	Balouchestan, Iranshahr	3BA	2345 Iranshahr, Ershad

Table 2. Morphological characters and their coding.

1- Habit: Shrubby=1, herbaceous=2
2- Rhizome: Absent=1, present=2
3- Stem branching: Absent=1, present=2
4- Stem: Erect=1, patent=2
5- Spine: Absent=1, present=2
6- Indumentum: Simple=1, stellate=2
7- Indumentum: Inclining=1, inclining and patent=2, patent=3, velotinous=4, tomentose=5
8- Density of indumentum: High=1, medium=2, low=3
9- Gland: Presence=1, Absence=2
10- Leaf shape: Ovate=1, elliptic=2
11- Leaf margin: Undulate to crenate=1, undulate to dentate=2, crenate=3, dentate =4
12- Leaf apex: Acute=1, acuminate=2, emariginate =3
13- Leaf base: Attenuate=1, oblique=2, cordate=3, cuneate=4
14- Leaf indumentum: Tomentose=1, sparse=2, glandular=3, stellate=4
15- Inflorescence: Corymb or raceme=1, few flower=2, cincinus=3, dense cincinus=4, racemose=5
16- Peduncle: Shorter or equal to pedicel=1, longer than pedicel=2
17- Pedicel indumentum: Absent=1, sparse=2, dense tomentose=3, stellate=4
18- Calyx indumentum: Pubescent=1, sparse or absent=2, dense=3, stellate=4, tomentose=5
19- Calyx spine: Absent=1, present=2
20- Corolla indumentum: Sparse=1, tomentose=2, glandular=3, stellate=4
21- Corolla color: White=1, violet=2, white and violet=3
22- Stamens: Free=1, connate=2
23- Stamen base indumentum: Absent=1, present=2
24- Anther opening: Pore=1, colpus=2
25- Style indumentum: Absent=1, present=2
26- Fruit color: Black=1, yellow to red=2
27- Seed surface: Reticulate=1, smooth=2, glandular=3
28- Plant height: cm
29- Leaf lenght: cm
30- Leaf width: cm
31- Petiole length: mm
32- Pedicel length: mm
33- Calyx length: mm
34- Corolla length: mm
35- Corolla diameter: mm
36- Anther length: mm
37- Filament length: mm
38- Style length: mm
39- Fruit diameter: mm
40- Seed diameter: mm

correlation matrix obtained among the operational taxonomic units (OTUs) using Euclidean distance as the similarity measure (Sheidai & Inamdar 1997). Grouping of all the sections was carried out by using ordination method based on principal component analysis (PCA) using correlation matrix (Chatfield & Collins 1995, Sheidai & Alishah 1998).

Karyotype analysis

Seeds were kept in water for 1-2 days, then stored in refrigerator (dark condition) for one week and finally brought to room temperature for germination. Freshly grown root tips were used for cytological preparations using 2 % aceto-orcein and 0.2 M 8-hydroxy quinolin (Sheidai & al. 1996). Chromosomes were identified according to Levan & al. (1964). Karyotypes were compared using total form percentage (Forni-Martin & al. 1994) and coefficient of variation (C.V) (Verma 1980). Symmetry of karyotypes was determined using Stebbins two way system (Stebbins 1971).

Grouping of the species included in the section *Solanum* having 2n = 48 chromosome number was carried out by single linkage clustering method using standardized karyological data (Sheidai & al. 1999).

Seed protein extraction, electrophoresis and data analysis

One hundred mg of each sample (25-50 seeds) was homogenised to obtain a fine powder. Proteins were extracted in pre-cooled mortar and pestle over ice with a 0.39 M Tris phosphate buffer (pH 8.3). The resulting mixture was centrifuged at 15,000 g for 10 min. The crude extracts were boiled for 5 min in 77 mM Tris-Hcl (pH 6.8), 4% sodium dodecyl sulphate (SDS), 10% 2-mercaptoethanol and 3% glycerol (Sanches-Yelamo & al. 1995). Protein electrophoresis by SDS-PAGE used 20 μg of protein in each lane. Vertical slab gels 1 mm thick were electrophoresed at a constant current of 30 mA for 8 hr. Coomassie Brilliant Blue G-250 was used for overnight gel staining followed by trichloro acetic acid as fixative.

To estimate species/population similarity as indicated by protein electrophoresis patterns, Jaccards, index was determined (Digby & Kempton

1994). Each protein band was considered as a qualitative character and coded as 1 (presence) versus 0 (absence) (Carrens & al. 1997). The resulting data matrix was used for cluster analysis and ordination based on principal components analysis. Statistical analysis used STATISTICA version 4.3 (1993) and SYSTAT version 6.0.1 (1996) software.

RESULTS AND DISCUSSION

Morphometry

Cluster analysis of morphological data among the species of section *Solanum* is presented in Fig. 1. Three clusters can be recognized at 6.8-linkage distance. The first cluster is comprised of different populations of *S. nigrum*. The second cluster is comprised of the *S. alatum* and *S. olgae* showing similarity to *S. nigrum*. The third cluster is comprised of different populations of *S. luteum* showing similarity to S. alatum.

Cluster analysis of the species included in the section *Dulcamara* is presented in Fig. 2. *S. dulcamara* and *S. persicum* showed more similarity than the other species, followed by *S. asiae-mediae* and *S. kieseritzkii*. High cophenetic correlation (>0.78) obtained, supported the clustering results.

Ordination of the species/populations on the first two principal components axes is presented in Fig. 3. Two sections of *Solanum* and *Dulcamara* from the subgenus *Solanum* are closer to each other than to the members of *Leptostemonum*; supporting the classical taxonomic treatment of the genus by Khatamsaz (1998).

Principal component analysis revealed that the first principal component comprises about 40% of total variance separating the two subgenera of *Solanum* and *Leptostemonum* while the second component comprises about 23% of total variance separating the two sections of *Solanum* and *Dulcamara* of the subgenus *Solanum*. The morphological characters which possessed high correlation (> 0.7) with the first principal component are presence/absence of spine; leaf, style and pedicel indumentum, calyx and petiole length and fruit diameter. Characters having high correlation with the second component are presence/absence of rhizome, type of stem, leaf base, corolla indumentum, seed surface and diameter. Therefore

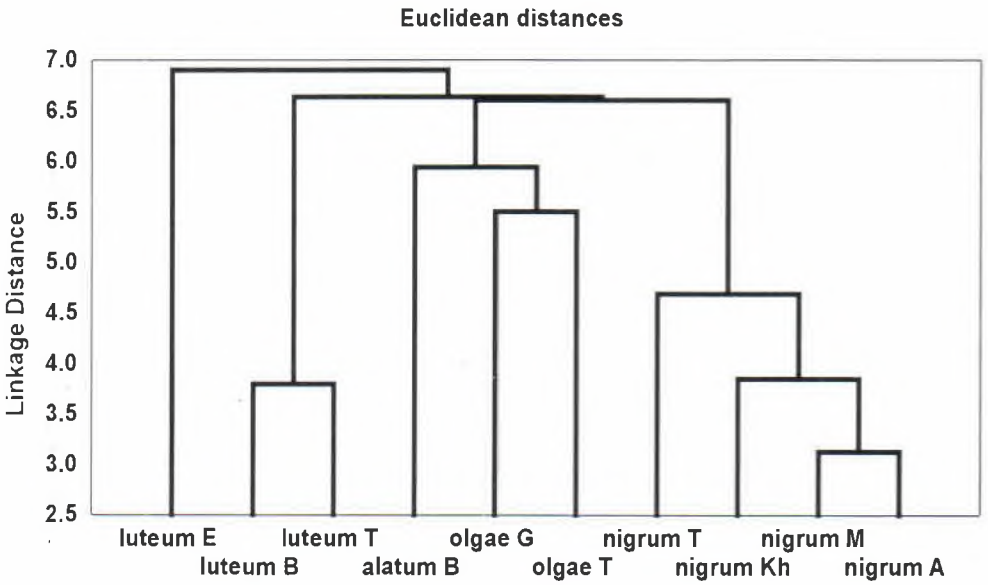


Fig. 1. Morphological cluster analysis (single linkage) in section *Solanum.* (populations abbreviations as in Table 1).

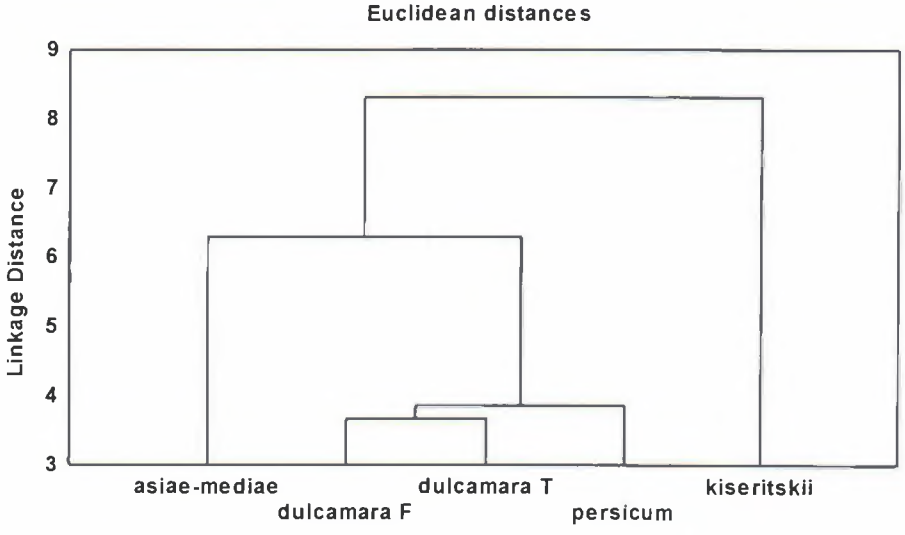


Fig. 2. Morphological cluster analysis (single linkage) in section *Dulcamara.* (populations abbreviations as in Table 1).

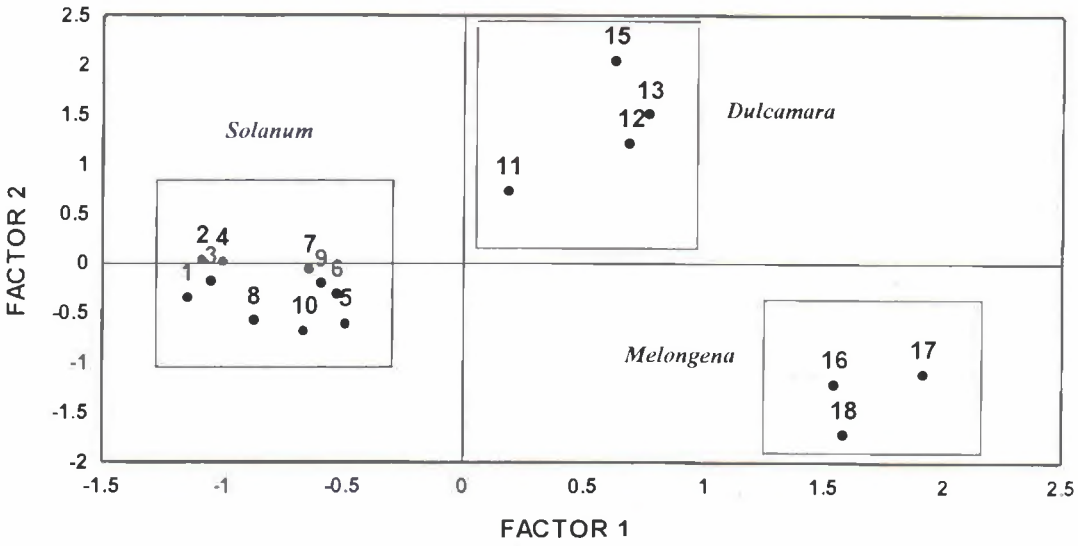


Fig. 3. PCA ordination of *Solanum* taxa based on morphological data. (sequence of taxa in Table 1).

these are the most variable morphological characters among the species studied.

Karyotype analysis

The somatic chromosome numbers are presented in Table 3 (Fig. 4). The species/ populations studied possessed 2n = 24 to 2n = 72. Considering x =12 as the basic chromosome number of the genus, the species of *S. incanum*, *S. surattense*, *S. dulcamara* and *S. persicum* are diploid (2n = 2x = 24). Azarbayejan and Tehran populations of *S. nigrum*, Bandar Abbas population of *S. alatum* and Balouchestan, Tehran and Esfahan populations of S. luteum are tetraploid (2n = 4x = 48). Mazandaran population of *S. nigrum* is hexaploid (2n= 6x = 72).

The present investigation reports the chromosome number of *S. persicum* for the first time (2n= 24) and supports the chromosome numbers reported for the other species (Rao & Kumar 1981, Ganapathi & Rao 1986, Trivedi & Sinha 1986, Anaso &Uzo 1990).

Variations observed in somatic chromosome number of the species/ populations studied indicate the role of polyploidy in the *Solanum*

Table 3. Karyotypic details of *Solanum* species (populations abbreviations as Table 1, Z=Zahedan, Sat= Number of SAT-chromosomes, TL=Total haploid chromatin lenght, L=Longest chromosome, S=Shortest chromosome, TF%=Total from percentage, C.V=Coefficient of variation, X=Mean chromatin length, KF=Karyotypic formulae).

Species	2n	Sat	T.L μm	L μm	S μm	T.F%	C.V	X μm	KF	Stebbins class
S.incanum	24	1	28.28	3.11	1.79	45.82	17.44	2.35	12m	1A
S. surattense	24	2	46.71	5.17	2.74	40.65	17.48	3.89	9m+3sm	1A
S. dulcamara	24	NO	39.57	4.02	2.60	44.65	12.15	3.29	12m	1A
S. persicum	24	1	29.45	3.09	1.71	43.42	15.51	2.45	11m+1sm	2A
S. nigrum (A)	24	NO	51.78	3.26	1.26	43.53	22.79	2.15	23m+1sm	1B
S. nigrum (T)	48	2	71.33	4.45	1.76	43.20	24.57	2.97	24m	1B
S nigrum (M)	72	5	99.86	4.62	1.47	44.29	23.82	2.77	36m	1B
S. alatum (B)	48	2	69.83	4.07	1.78	43.13	20.34	2.90	23m+1sm	1B
S. alatum (Z)	48	NO	70.84	4.12	1.95	43.70	19.32	2.95	23m+1sm	1B
S. luteum (B)	48	2	65.28	4.22	1.64	43.18	22.79	2.72	23m+1sm	1B
S. luteum (T)	48	2	71.83	4.34	2.01	42.82	20.06	2.99	22m+2sm	1B
S. luteum (E)	48	2	60.09	3.79	1.56	43.50	23.20	2.50	24m	1B

speciation/populations adaptation. Polyploidy has been reported for *S. nigrum* and *S. incanum* by others too (Rao & Kumar 1983, Anaso & Uzo 1990).

Details of the karyotypes are presented in Table 3. Among diploid species, the highest total chromatin length occurred in *S. nigrum* (51.78 μm) and the least in *S. incanum* (28.28 μm). The highest value for length of the longest chromosome occurred in *S. surattense* (5.17 μm), while the least value occurred in *S. persicum* (3.09 μm). Among tetraploids the highest total chromatin length was observed in *S. luteum* Tehran (71.83 μm) and the least in *S. nigrum* Azarbayejan (51.78 μm).

Among diploids *S. nigrum* possessed the highest C.V value (22.79) showing high variability in its karyotype, while *S. dulcamara* possessed the lowest value (12.15). Among tetraploid species the highest C.V value occurred in *S. nigrum*

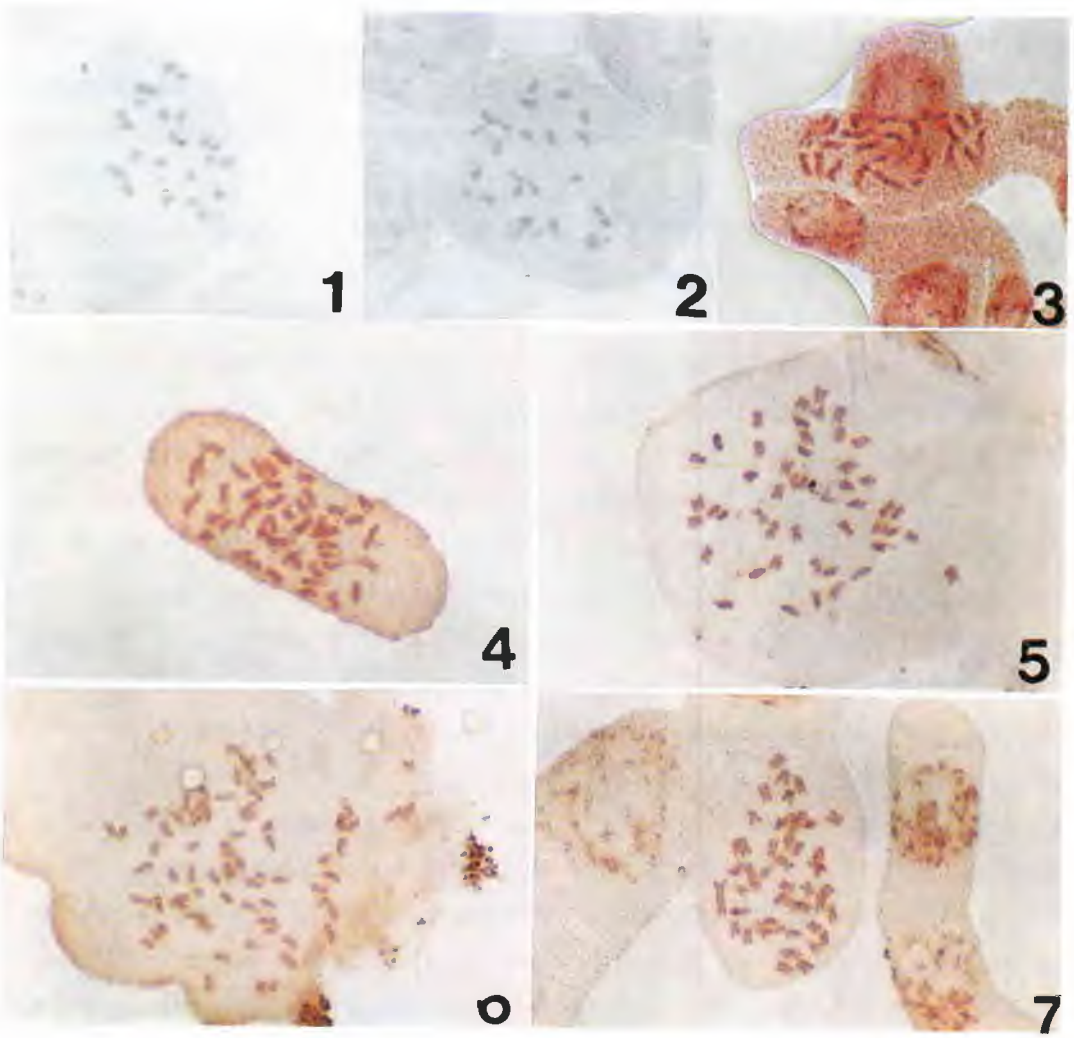


Fig. 4. Representative photographs of somatic chromosomes in the *Solanum* taxa 1- *Solanum persicum*, 2- *S. incanum*, 3- *S. surattense*, 4- *S. nigrum* (Tehran), 5- *S. luteum* (Esfahan), 6- *S. nigrum* (Mazandaran), 7- *S. alatum* (Bandar Abbas) Magnifications X 820.

Tehran (24.57) and the least in *S. alatum* Zahedan (19.32).

Variations were observed in the number of SAT-chromosomes and the chromosomes carrying secondary constriction among the species/populations studied (Fig. 5). For example although all three populations of *S. luteum* possessed two SAT-chromosomes, in Bandar Abbas population chromosome 9 and 18 are SAT-chromosomes, in Tehran population chromosomes 3 and 20 and in Esfahan population chromosomes 2 and 11 are SAT-chromosomes. SATs were present on the short as well as long arm of the chromosomes (Fig. 5).

Karyotypic formulae and symmetry are presented in Table 3. The chromosomes were mostly of m type with few sm. Diploid woody species occupied the primitive classes of the Stebbins system (1A and 2A), while tetraploid and hexaploid herbaceous taxa occupied class 1B, which is more asymmetrical. Therefore it seems that during species diversification and changing from woody to herbaceous habit, an increase in karyotype asymmetry has been occurred along with change in chromosome number and increase in total chromatin length as the herbaceous species studied possess higher value of total chromatin length.

Variations observed in the species karyotypic formulae, indicates occurrence of structural changes in the chromosomes during speciation. Pearson coefficient of correlation determined for the karyotypic parameters supports this idea. Higher value (>0.90) was obtained for the length of total chromosomes among diploid and tetraploid species separately, but a lower r value (<0.80) was obtained for long and short arms and still lower value (even negative value) (<0.60) for S/L ratio.

Cluster analysis of the species included in the section *Solanum* based on karyotypic data is presented in Fig. 6. Populations of *S. alatum* show similarity to each other and *S. alatum* Bandar Abbas show relationship with *S. luteum* Tehran. *S. nigrum* Tehran and Azarbayejan show similarity to different populations of *S. luteum* supporting the morphological clustering results (Fig. 1). High cophenetic value (>0.80) obtained for cluster analysis indicates fit of the

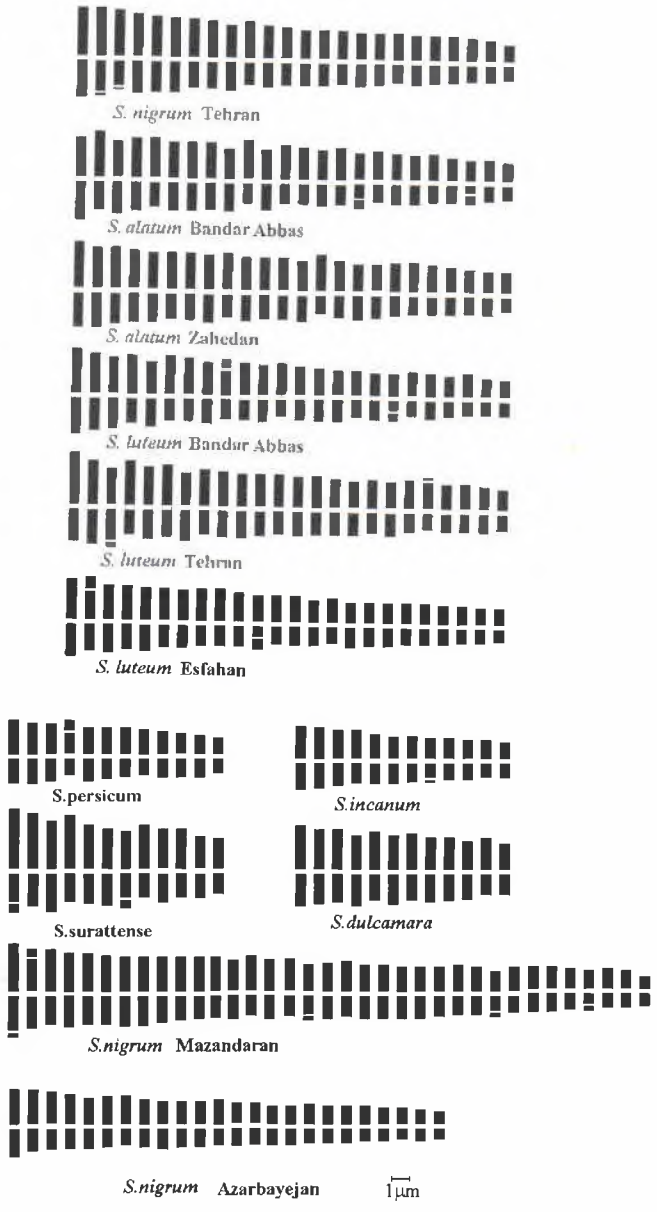


Fig. 5. Idiograms of the *Solanum* species.

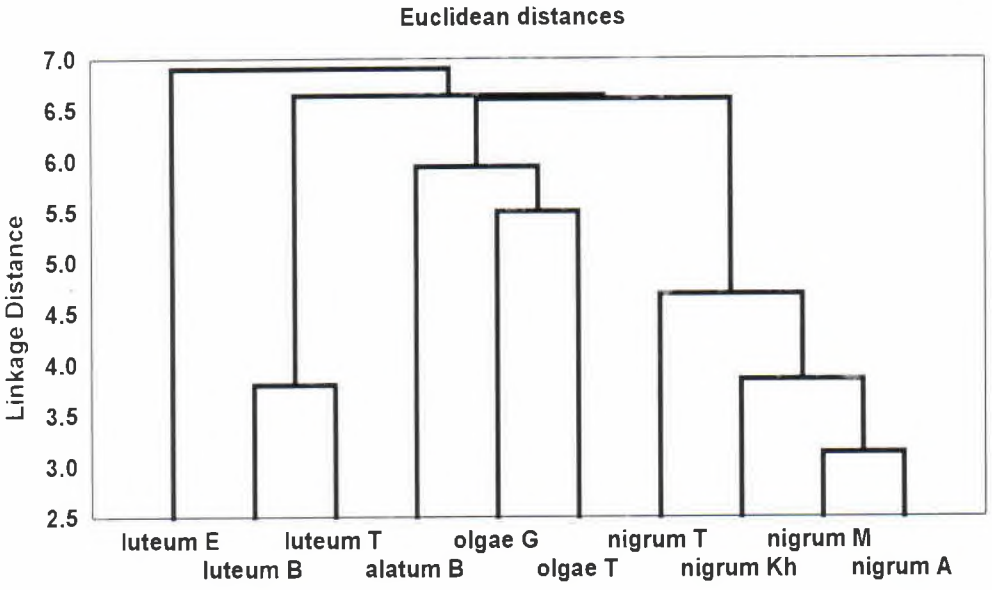


Fig. 6. Single linkage cluster analysis of karyotypic data in section *Solanum*. (populations abbreviations as in Table 1).

clusters to the original data (Rohlf 1987).

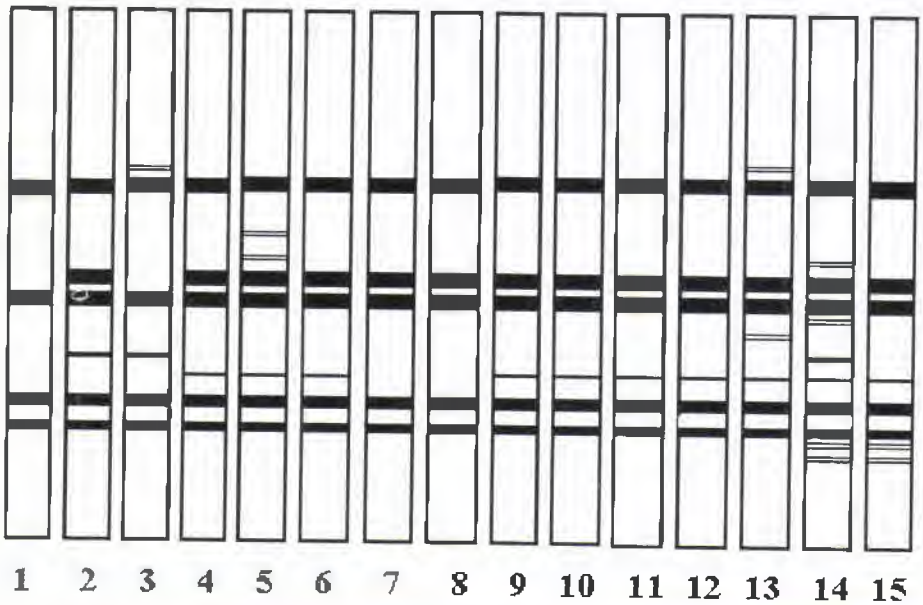
Seed protein analysis

The bands obtained from SDS-PAGE analysis are presented in Table 4 (Figs. 7 & 8). In total 14 bands were obtained. Bands 2, 6, 11 and 12 are present in all the species/populations. Band 3 is present in Bandar Abbas population of *S. luteum*, band 7 is present only in *S. incanum* and band 8 is only in Fars population of *S. dulcamara*. Therefore these bands may be considered as species-specific.

Cluster analysis of protein data and ordination based on the first two principal components axes are presented in Figs. 9 and 10. In the phenogram obtained from cluster analysis two major clusters are formed at 0.4-linkage distance separating subgenus *Solanum* from Leptostemonum. The first cluster is

Table 4. SDS-PAGE protein bands in *Solanum* species (populations abbreviations as table 1.)

Species	Band													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
S. dulcamara T	0	1	0	0	0	1	0	0	0	0	1	1	0	0
S. persicum	0	1	0	0	1	1	0	0	1	0	1	1	0	0
S. kiesritzkii	1	1	0	0	0	1	0	0	1	0	1	1	0	0
S. luteum E	0	1	0	0	1	1	0	0	0	1	1	1	0	0
S. luteum B	0	1	1	1	1	1	0	0	0	1	1	1	0	0
S. luteum T	0	1	0	0	1	1	0	0	0	1	1	1	0	0
S. alatum B	0	1	0	0	1	1	0	0	0	0	1	1	0	0
S. olgae	0	1	0	0	1	1	0	0	0	0	1	1	0	0
S. nigrum KH	0	1	0	0	1	1	0	0	0	1	1	1	0	0
S. nigrum T	0	1	0	0	1	1	0	0	0	1	1	1	0	0
S. nigrum M	0	1	0	0	1	1	0	0	0	1	1	1	0	0
S. nigrum A	0	1	0	0	1	1	0	0	0	1	1	1	0	0
S. dulcamara F	1	1	0	0	1	1	0	1	0	1	1	1	0	0
S. incanum	0	1	0	1	1	1	1	0	1	1	1	1	1	1
S. surattense	0	1	0	0	1	1	0	0	0	1	1	1	1	1



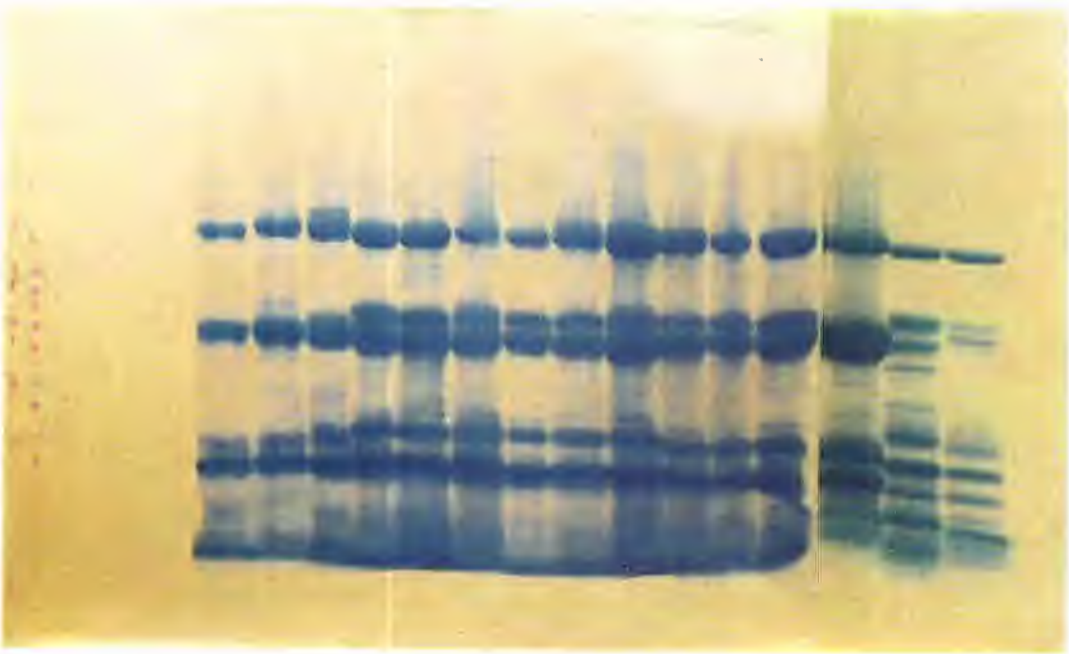


Fig. 7 & 8. SDS-PAGE protein bands in *Solanum* species, sequence of taxa as in table 4.

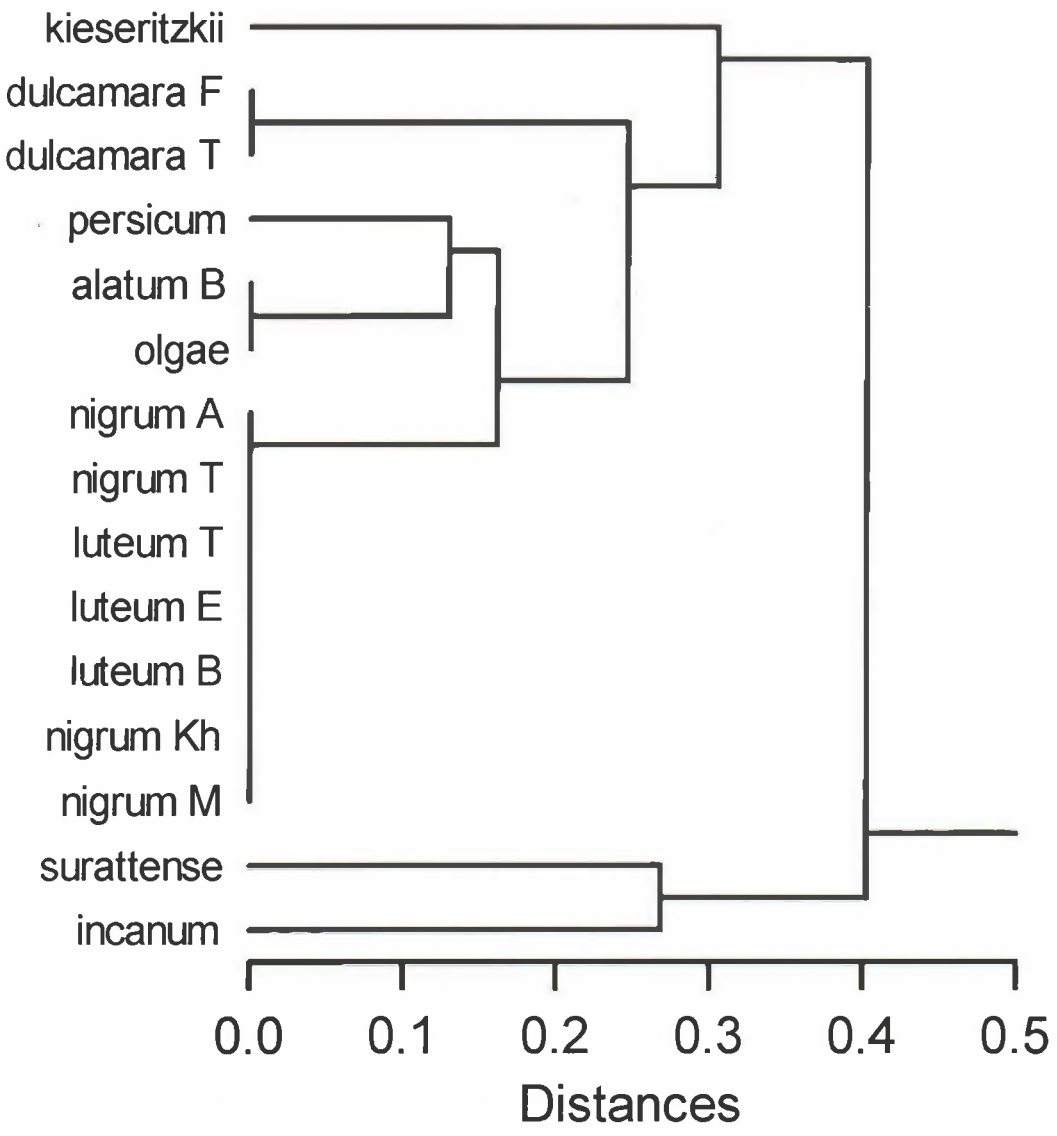


Fig. 9. G. Ward cluster analysis of protein data (populations abbreviations as in table 1).

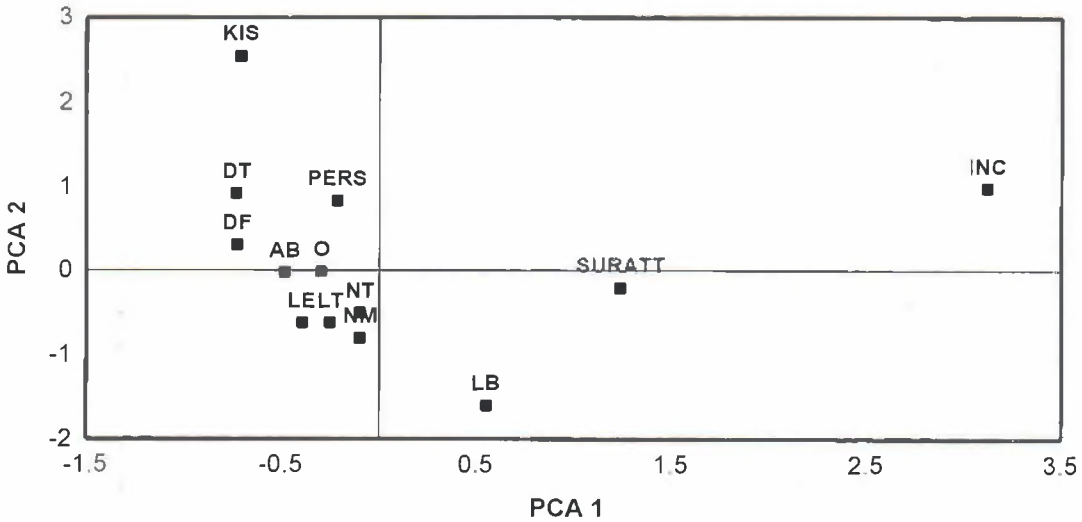


Fig. 10. Ordination of *Solanum* species based on protein data, KIS=*kieseritskii,* PERS =*persicum,* INC= *incanum*, SURATT= *surattense,* DT= *dulcamara* Tehran, DF= *dulcamara* Fars, *AB*= *alatum* Bandar Abbas, O= *olgae,* LE= *luteum* Esfahan, LT= *luteum* Tehran, LB= *luteum* Bandar Abbas, NM = *nigrum* Mazandaran, NT= *nigrum* Tehran (*S. nigrum* Azarbayjan & Khozestan are not shown as are overlapped with the other *S. nigrum* populations).

comprised of *S. surattense* and *S. incanum* from the subgenus *Leptostemonum*, while the second major cluster is comprised of the species *S. kieseritzkii*, S. *dulcamara*, *S. persicum*, *S. alatum*, *S. olgae*, *S. nigrum* and *S. luteum* included in the subgenus *Solanum*. Three sub-clusters are formed in this major cluster. The first sub-cluster is comprised of different populations of *S. nigrum* and *S. luteum* showing inter-relationship. This was earlier shown by cluster analysis of morphological (Fig. 1) and karyotypic data (Fig. 6). The second sub-cluster is comprised of *S. alatum* Bandar Abbas, *S. olgae* and *S. persicum*, in which the first two species show more similarity, supported by morphological analysis.

The third sub-cluster is comprised of Tehran and Fars populations of *S. dulcamara* to which *S. kieseritzkii* is joined with some distance. Separation of sections *Solanum* and *Dulcamara* is evident in ordination based on principal components analysis (PCA) of protein data too (Fig. 10). The first PCA which comprises about 34% of the total variance, separates the two subgenera of *Solanum* and *Leptostemonum*, while the second PCA which comprises about 21% of total variance, separates the two sections *Solanum* and *Dulcamara*.

Principal components analysis (PCA) of protein bands revealed that the first four PCA axes comprise about 64% of the total variance. The most variable protein bands of the first PCA component are bands 4, 7, 13 & 14, while bands 1 & 9 are the most variable bands of the second component (Fig. 11). Bands 8 and 3 are the most variable bands of the third and fourth components respectively. Therefore it seems that the genes coding these bands might have changed during speciation.

In short the main findings of the present study can be summarized as: The somatic chromosome number of *S. persicum* is a new report, polyploidy and structural changes of chromosomes have played role in the speciation of the *Solanum*, numerical taxonomy and seed protein analysis supports the classical taxonomic treatment of the genus *Solanum* and provides evidence for the species inter-relationship.

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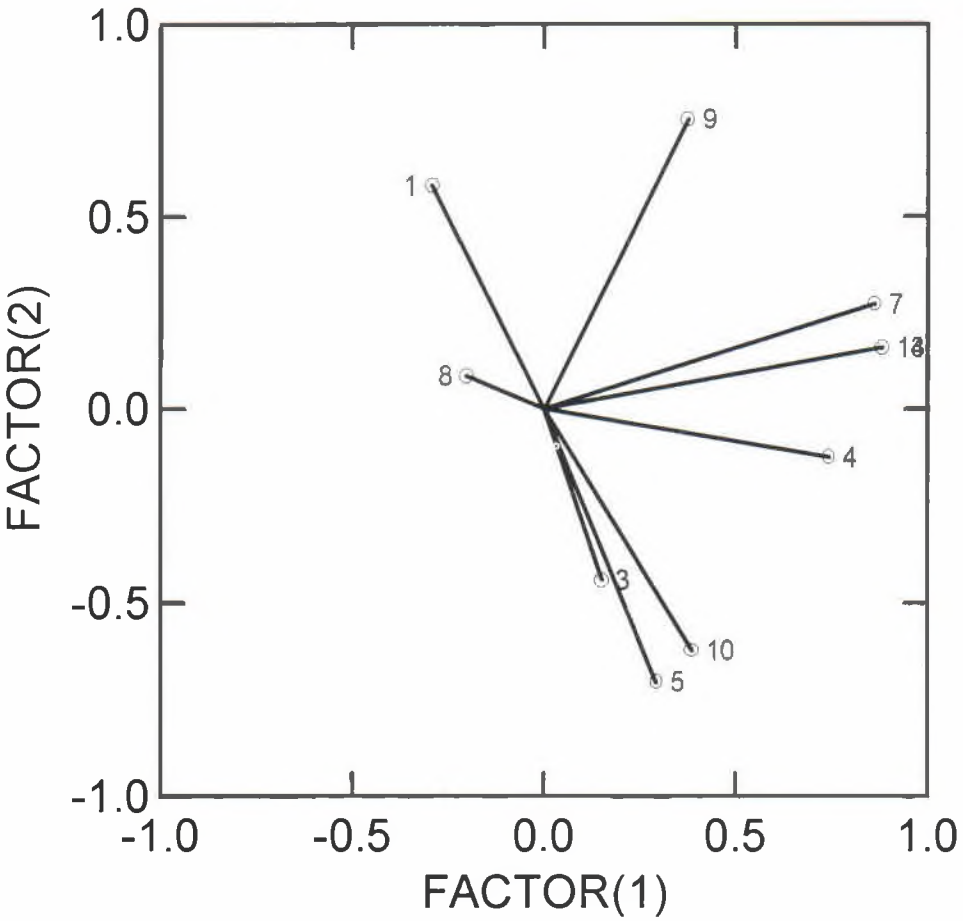

Fig. 11. Factor loading of protein data showing the most variable bands of the first two PCA factors.

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