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Karyotypic study in some Iranian species and populations of *Tulipa* L. (Liliaceae)

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Abstract - Karyotypic studies were performed on 12 species/ populations of Iranian tulips for the first time. The species/ populations possessed $2n = 2x = 24$ chromosome number but varied in details of karyotype including type of chromosomes, karyotype symmetry and number of SAT-chromosomes and differed significantly in total chromatin length as well as length of chromosome arms. A pair of heteromorph chromosomes occurred in *T. humilis*. The use of karyotypic data in taxonomy of the genus *Tulipa* L. is discussed.

Key words: Cluster analysis, Heteromorph chromosomes, Iran, Karyotype, Tulips.

INTRODUCTION

Tulips (*Tulipa* L.) are among important plants widely used as ornamentals, they have been originated in Eastern countries and via Iran and Turkey were introduced in Europe (MATIN 1998). The number of Tulip species occurring in Iran varies according to different authors. BOISSIER (1882) reported seven *Tulipa* species from Iran while WENDELBO (1977) reported 12 species, RECHINGER (1990) reported 19 and Parsa reported 23 species (MATIN 1998).

Tulipa species are distributed in two sub-genera namely Eriostemones, including three sections and Tulipa, including five sections (RAAMSDONK and VARIES 1995). Several species belonging to these sections occur in Iran. Due to economic importance of tulips, they have been subjected to extensive cytogenetical studies in other countries (for example ATHANASIOU 1988; JOHNSON and BRANDHAM 1977), but there has been no report from Iran.

The present paper is a part of biosystematic study in *Tulipa* of Iran reporting karyotypic features in some of the species for the first time trying to present the chromosome number of these species and elucidate the karyotypic changes during the species diversification. It is also attempted to reveal the use of such data in delimiting the species studied.

MATERIALS AND METHOD

Karyotypic studies were performed in 12 populations of seven *Tulipa* species/ varieties from two subgenera of 1) subg. Eriostemones including *Tulipa biflora* Pall and *T. sogdiana* Bunge from the sec. Biflores as well as *T. humilis* Herbert from the sec. Sazatiles; 2) subg. Tulipa including *T. montana* Lindl. var. *montana*, *T. montana* L. var. *chrysantha* (Boiss.) from the sec. Clusianae, *T. systola* Stapf. and *T. boogiana* B. Fedtsch. from the sec. Tulipanum.

Fresh roots obtained from collected healthy bulbs were used for cytological preparations using 2% aceto-orcein and 0.2 M 8-hydroxy quinolin (SHEIDAI *et al.* 2000b). Chromosomes were identified according to LEVAN *et al.* (1964). Karyotypes were compared using coefficient of variation (CV; VERMA 1980), total form percentage (TF%; HUZIWARA 1962), Stebbins' two

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way system of karyotype symmetry (STEBBINS 1971) and Romero-Zarco indices (ROMERO ZARCO 1986).

In order to show quantitative differences among different populations of a single species, factorial analysis (ANOVA) was performed on the size of the chromosomes, long arms as well as short arms, using the chromosomes and the species/ populations as the two factors (SHEIDAI *et al.* 2000a). In order to compare the present findings and those reported by the other authors on similar species or closely related ones and due to difference in cytological preparation, the relative values of karyotypic features were used.

For grouping the species/ populations having similar karyotypic features, relative data were standardized (mean = 0, variance = 1) used in cluster analysis and ordination based on principal components analysis (PCA) (SHEIDAI *et al.* 2000b). Euclidean/ squared Euclidean distance was used as a measure of similarity in cluster analysis. In order to identify the most variable karyotypic characters among the species studied, factor analysis was performed (SHEIDAI *et al.* 1999).

Multivariate statistical analyses used SPSS ver. 9. (1988) software.

RESULTS AND DISCUSSION

Geographical distribution of *Tulipa* species/ populations studied is presented in Fig. 1 and their somatic chromosome number as well as karyotypic datails are presented in Figs. 2 and 3 and Tables 1 and 2. All the species and popula-

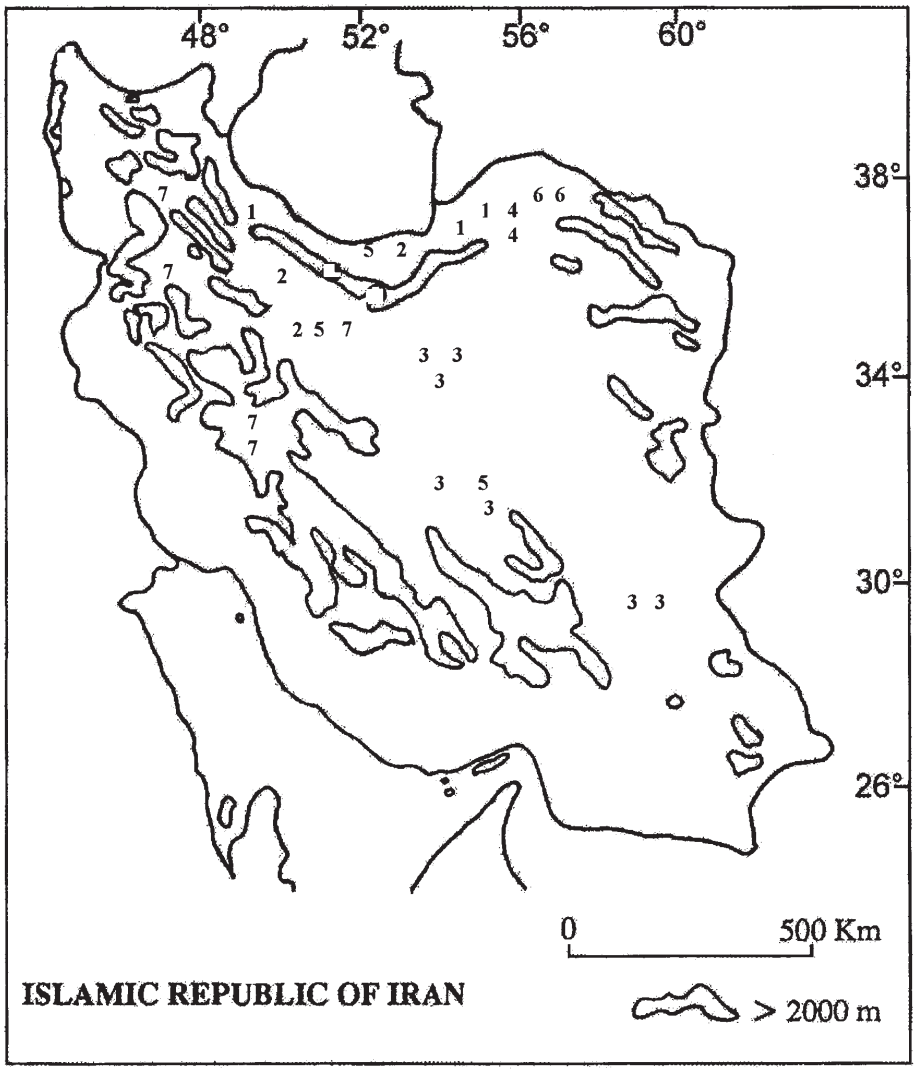


Fig. 1 – Geographical distribution of *Tulipa* species studied. 1 = *Tulipa montanum* var. *montanum*, 2 = *T. montanum* var. *crusantha*, 3 = *T. biflora*, 4 = *T. sogdiana*, 5 = *T. humilis*, 6 = *T. hoogiana*, 7 = *T. systola*.

tions studied possessed $2n = 24$ chromosome number supporting the earlier reports (UPCOT and LA COUR 1936; RAAMSDONK and VRIES 1992, 1995). The basic chromosome number of tulips is x = 12, therefore the species/ populations studied are diploid. However triploid ($2n = 36$), tetraploid ($2n = 46$) as well as pentaploid ($2n = 60$) species are also known (RAAMSDONK and VRIES 1992, 1995). The occurrence of polyploidy among tulips is considered as a mean for adaptation to environmetal variations (BOTSCHANTZEVA 1982).

Although the species/ populations studied possessed similar chromosome number, they differed in details of karyotypes including chromatin length, karyotypic formulae and the number of SAT-chromosomes (Table 2).

Among *T. montana* populations, the lowest value of total haploid chromatin length (133.8 µm) occurred in Emamzadeh Hashem while the highest value (153.9 µm) occurred in Abali population. Jajerood population possessed the highest value of longest chromosome (20.6 µm) while Emamzadeh-Hashem population possessed the lowest value for the same (15.5 µm). The highest and lowest values for the smallest chromosome occurred in Abali (7.0 µm) and Jajerood (6.0 µm) populations respectively.

Jajerood population possessed the highest value of CV (32.13) showing the highest variation among its chromoromes compared to the other *T. montana* populations while Darabad populatoin possessed the lowest value (28.56). These populations varied in their karyotypic formulae too (Table 2), the chromosomes were mostly of telocentric and sub-telocentric and 1-2 sub-metacentric chromosomes except in Jajerood population of var. *chrysantha* which possessed only st and t chromosomes.

Variation in karyotypic formulae of the populations studied indicate the occurrence of structural changes in the chromosomes which is supported by statistical analyses (following paragraphs).

T. montana populations studied occupied 4B class of Stebbins classification which is among advanced classes of karyotype symmetry (more asymmetrical). Jajerood population due to its

Table 1 – Karyotypic details of *Tulipa* species/populations.

Species/ Population	Locality	Voucher No.	$2n$	x	TL µm	L µm	S µm	L/S µm	X µm
Tulipa var. *montana*	*montana* Golestan	99102	24	2x	147.8	17.5	6.7	2.6	12.3
T. montana var. *montana*	Emamzadeh-Hashem	99105	24	2x	133.8	15.5	6.1	2.5	11.1
T. montana var. *montana*	Abali	99108	24	2x	153.9	20.0	7.0	2.8	12.8
T. montana var. *crysantha*	Darabad	99104	24	2x	147.6	17.5	6.4	2.7	12.3
T. montana var. *crysantha*	Jajerood	99109	24	2x	151.2	20.6	6.0	3.4	12.6
T. humilis	Touchal	99131	24	2x	162.1	20.3	9.4	2.1	13.5
T. humilis	Hamedan	99125	24	2x	198.6	22.6	11.4	1.9	16.5
T. biflora	Tehran	2000-5	24	2x	217.1	24.4	13.4	1.8	18.1
T. sogdiana	Semnan	2000-7	24	2x	181.3	22.7	10.0	2.2	15.1
T. hoogiana	Golestan	99134	24	2x	184.7	20.8	10.3	2.0	15.4
T. systola	Bamu	2000-2	24	2x	198.4	22.3	11.7	1.9	16.9
T. systola	Darabad	99139	24	2x	198.4	22.3	11.7	1.9	16.5

Abbreviations: x = Ploidy level, TL = Total haploid chromatin length, L = Longest chromosome, S = Shortest chromosome, L/S = Longest/ shortest chromosome, X = Mean chromatin length.

high A1 value (0.86) and low TF% (13.70) possesses the most symmetrical karyotype while Golestan population with a lower A1 value (0.82) and higher TF% (17.70) possesses the most asymmetrical karyotype.

Pearson coefficient of correlation determined for karyotypic parametrs revealed a high value ($r > 0.90$) for total chromatin length and long arm of the chromosomes, indicating homogenity of the karyotypes and inclusion of these varieties in the same species. However a lower value of correlation for short arm of the chromosomes ($r < 0.60$) and arm ratio ($r < 0.70$) indicates changes in the short arms as well as arm ratios due to structural changes of the chromosomes (SHEIDAI *et al*. 2000b).

ANOVA test performed on the size of chromosomes, long arms as well as short arms among *T. montana* populations showed presence of significant difference ($p < 0.05$) in the size of short arms only. This may indicate that during varieties differentiation or population adaptation, no significant quantitative change has occurred in total and the long arm length of chromosomes, but a significant change has occurred in the short arms length.

The variations observed in karyotypic details of the two varieties and their populations as well as lack of a significant difference in the size of chromosomes indicate that karyotypic features may not help in differentiating the varieties from each other and morphological characters are more helpful.

Among the other species studied Bamu population of *T. systola* possessed the highest mean total chromatin length (202.33 mm) and the lowest value occurred in Tochal population of *T. humilis*. The highest value for longest chromosome occurred in *T. biflora* (24.40 mm) while the lowest value occurred in Touchal population of *T. humilis* (20.37 mm).

The highest value of CV occurred in *T. sogdiana* (24.76) and the lowest value occurred in *T. biflora* (18.68). Both values are higher than those observed in the populations of *T. montana* var. *montana* and var. *crysantha*. Therefore these kary-

Table 2 – Karyotypic formulae and symmetry (localities as in Table 1).

Species	SAT No.	SAT-ch	TF%	CV	KF	ST-class	A1	A2
T. montana var. *montana*	NO	NO	17.70	32.70	1sm + 9st + 2t	4B	0.82	0.33
T. montana var. *montana*	NO	NO	15.70	28.70	2sm + 7st + 3t	4B	0.83	0.29
T. montana var. *montana*	NO	NO	16.60	31.10	1sm + 10st + 1t	4B	0.83	0.31
T. montana var. *crysantha*	NO	NO	15.80	28.56	1sm + 8st + 3t	4B	0.83	0.29
T. montana var. *crysantha*	NO	NO	13.70	32.13	7st + 5t	4B	0.86	0.32
T. humilis	NO	NO	23.10	24.20	1sm + 11st	4B	0.68	0.20
T. humilis	3	1, 2, 3	19.80	20.30	2sm+ 10st	4A	0.72	0.20
T. biflora	2	2, 6	21.10	18.60	2sm+ 10st	4A	0.72	0.18
T. sogdiana	1	5	20.82	24.76	3sm+ 9st	4B	0.72	0.24
T. hoogiana	2	11, 4	20.85	19.68	1sm+ 11st	4B	0.75	0.19
T. systola	NO	NO	18.89	18.73	12st	4A	0.74	0.19
T. systola	NO	NO	19.98	19.29	12st	4A	0.76	0.18

Abbreviations: SAT No. = Number of SAT chromosmes, SAT-ch = Chromosomes carrying secondary constriction, TF% = Total form percentage, CV = Coefficient of variation, KF = Karyotypic formulae, ST-class = Stebbins' class, NO = not observed.

otypes show more variatoin in the size of chromosomes compared to those of *T. montana* varieties.

Pearson coefficient of correlation determined for karyotypic parameters among the species studied revealed a high value (r >0.90) for total chromatin length and long arm of the chromosomes, indicating homogenity of the karyotypes and inclusion of the species in the same genus. However a lower value of correlation for short arm of the chromosomes (r = 0.10 - 0.60) and arm ratio (r = 0.40 - 0.70) indicates changes in the short arms as well as arm ratios due to structural changes of the chromosomes.

ANOVA test performed on karyotypic parameters among different species studied revealed

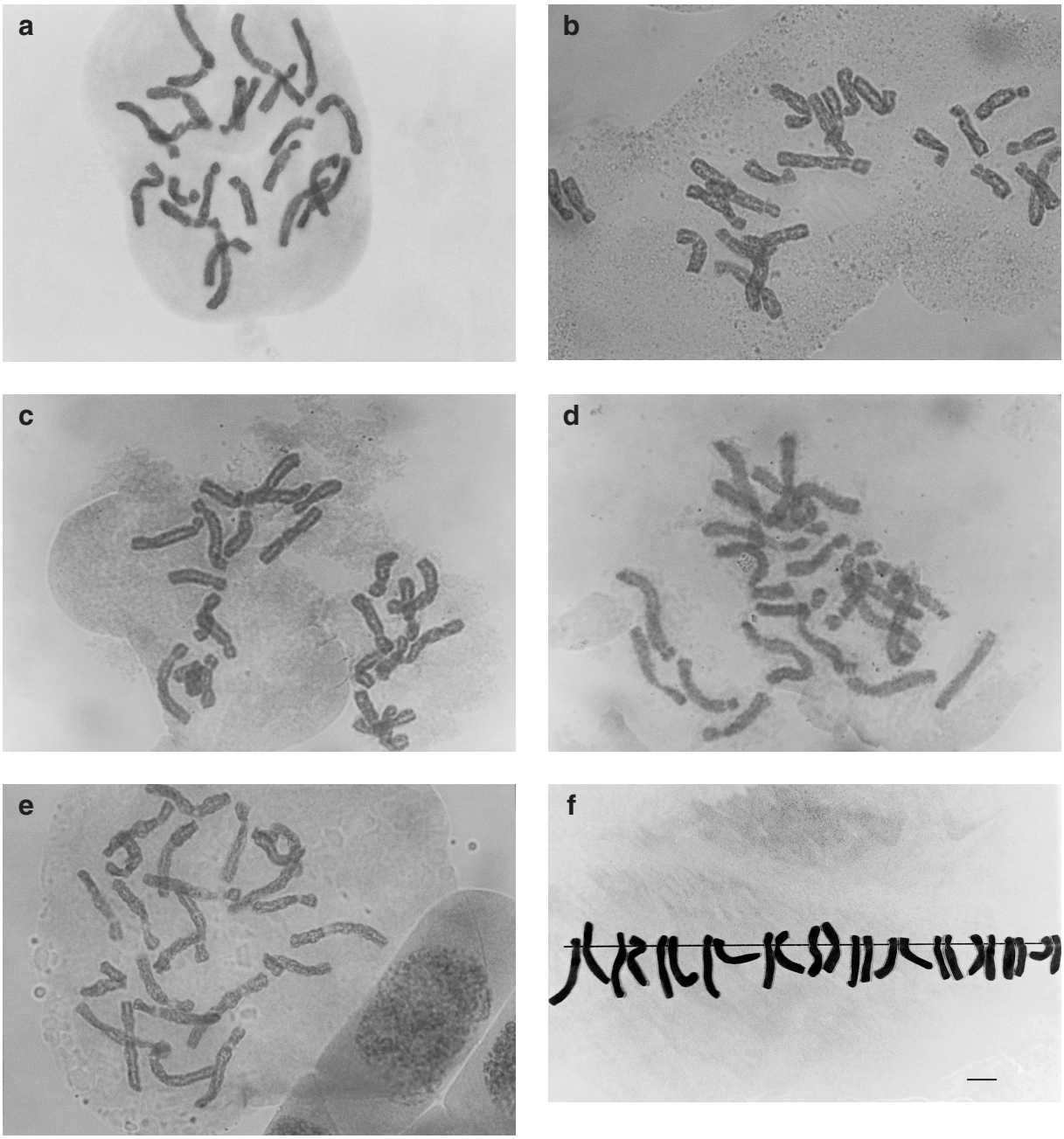


Fig. 2 – Representative somatic cells and karyogram in *Tulipa* species. a = *Tulipa humilis* Hamedan, b = *T. hoogiana* Gorgan, c = *T. sogdiana* Semnan, d = *T. biflora* Tehran, e = *T. systola* Darabad, f = karyogram of *Tulipa humilis* Hamedan showing a heteromorph bivalent. Scale = 10 μm.

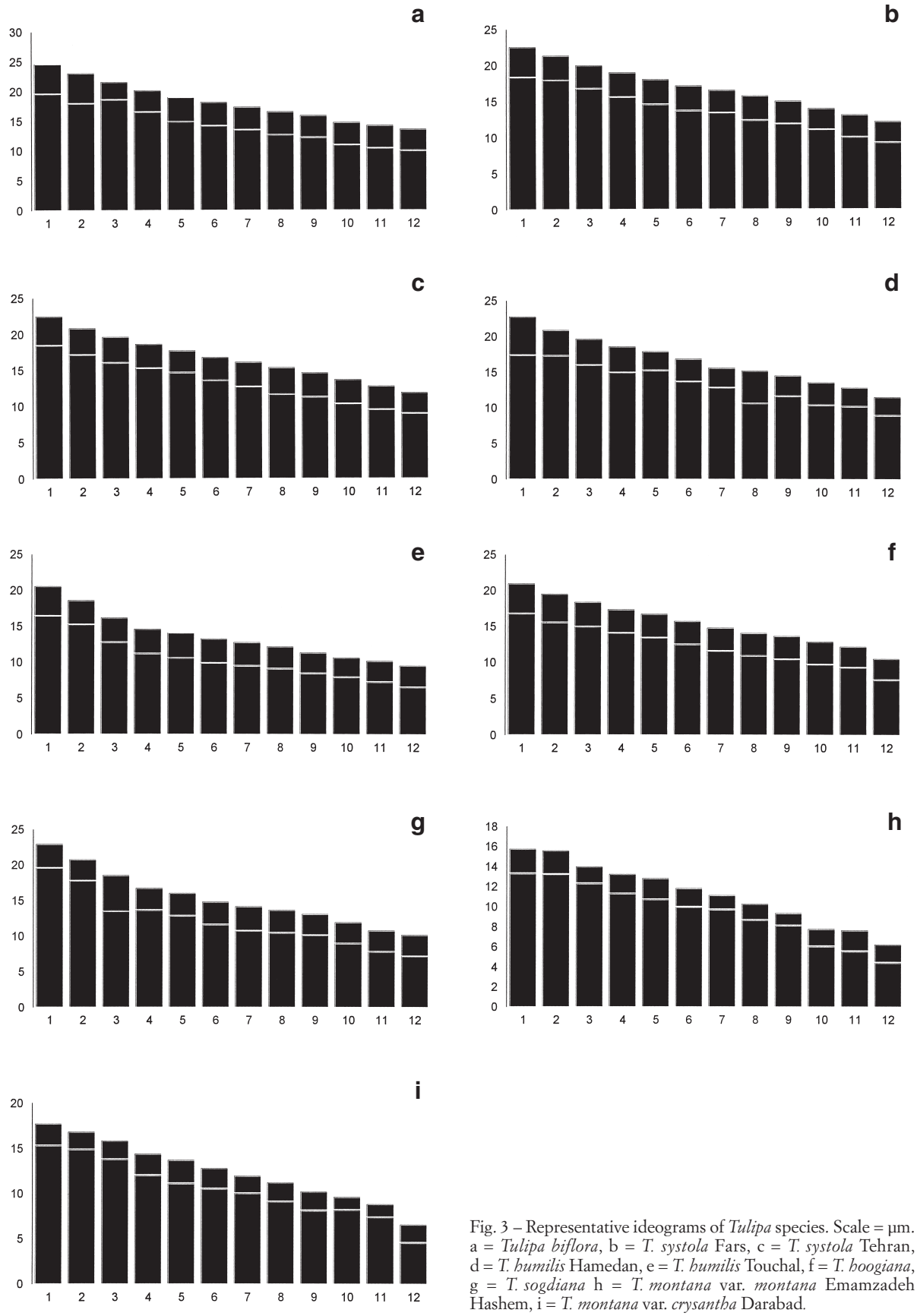


Fig. 3 – Representative ideograms of *Tulipa* species. Scale = µm. a = *Tulipa biflora*, b = *T. systola* Fars, c = *T. systola* Tehran, d = *T. humilis* Hamedan, e = *T. humilis* Touchal, f = *T. hoogiana*, g = *T. sogdiana* h = *T. montana* var. *montana* Emamzadeh Hashem, i = *T. montana* var. *crysantha* Darabad.

the presence of a significant difference ($p < 0.05$) in total chromosome length, long arms of the chromosomes as well as arm ratios. A significant difference ($p < 0.05$) in the size of short arms of the chromosomes occurred only between *T. biflora* and the other species studied. Therefore karyotypic changes during *Tulipa* speciation has been accompanied with significant chromatin changes (either loss or gain; quantitative change) as well as qualitative change as is revealed in difference in karyotypic formulae and arm ratios. This is also supported by differences observed in the karyotypic formulae and SAT- chromosomes (number of SATs and the chromosomes carrying secondary constriction) in the species studied (Table 2).

In order to compare the present findings with those reported from the other species of the subg. Tulipa from former USSR (BOCTSCHANZEVA 1982), the relative values of karyotypic features were used. Stebbins, two way system of karyotype classification revealed that in the subg. Tulipa, *T. systola* and *T. hoogiana* from the section Tulipanum occupy 4A and 4A and 4B respectively, *T. montana*, *T. wilsoniana* and *T. linifolia* from the section Clusianae occupy 4B class while *T. lehmanniana* (sec. Kolpakovskianae), *T. micheliana* (sec. Eichler) and *T. scherenki* (sec. Gesnerianae) occupy 3B class. These three sections are also considered close to each other based on their morphological characetrs (MATIN 1998).

In the subg. Eriostemons (sec. Biflores), *T. biflora* occupies 3A class while *T. sogdiana* occupies 4B class. Therefore *T. sogdiana* possesses a more advanced (asymmetrical) karyotype compared to *T. biflora*. Populations of *T. humilis* occupy 4A and 4B classes. Therefore in general *Tulipa* species (from both sub-genera) possess asymmetrical karyotype as they occupy mostly 4A and 4B classes of Stebbins, two way system of karyotype classification.

From the present results it seems that increase in the karyotype asymmetry is accompanied with the loss of chromatin material as the species with more asymmetric karyotype (4B class) possess significantly shorter total chromatin length compared to the species with less asymmetric karyotype (4A class). Therefore chromatin loss may be one of the possible karyotypic changes during the species diversification in the genus *Tulipa*.

Cluster analysis and ordination of the species/ populations from the subg. Tulipa based on the relative karyotypic data produced similar results (Figs. 4 and 5), indicating distinctness of the species studied as they stand in separate groups. This is also supported by ANOVA test performed on the size of chromosomes as well as chromosome arms, indicating a significant difference among the species studied.

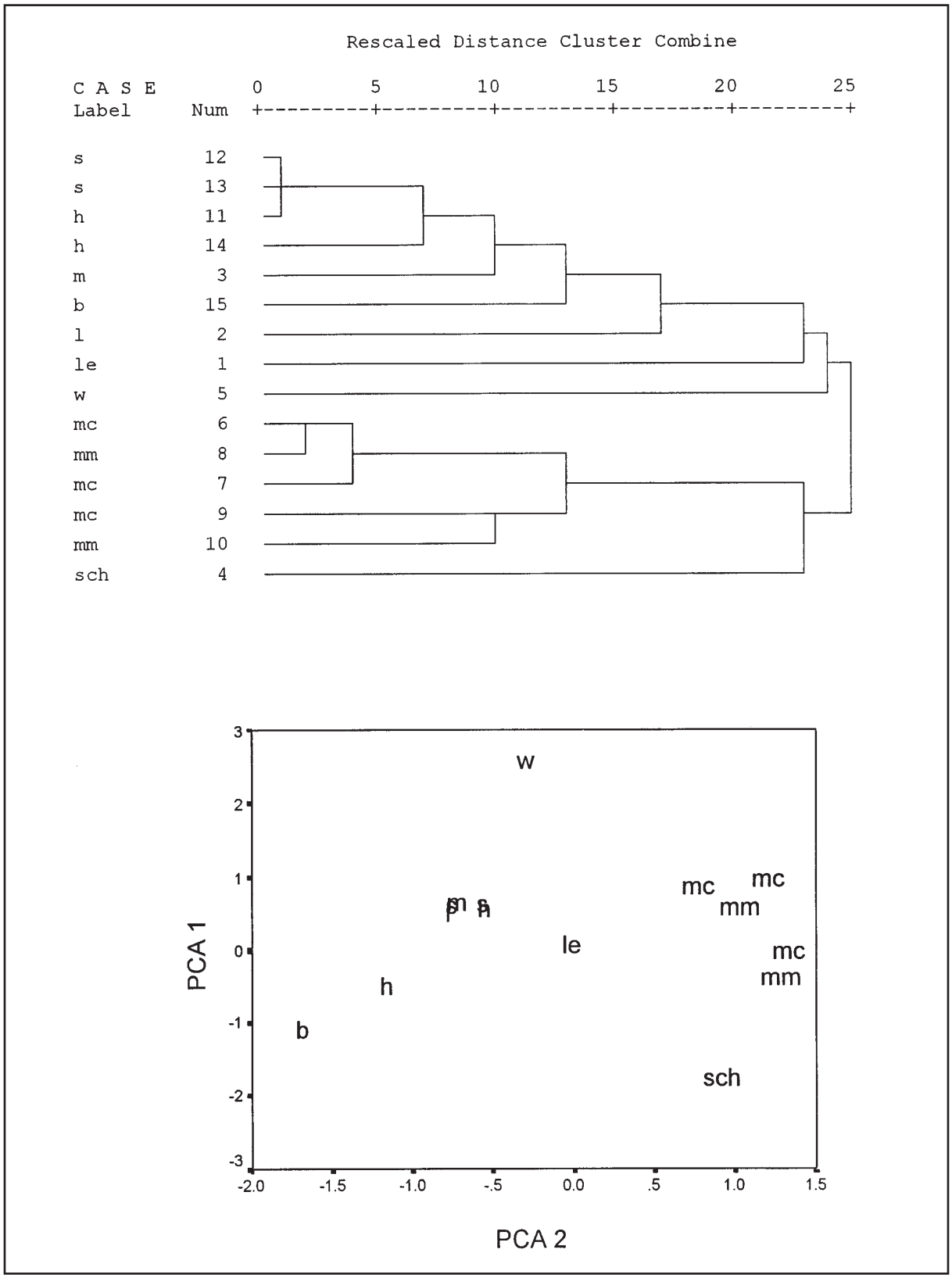
An interesting result is the separation of *T. montana* from *T. linifolia* which are very similar in morphological characters but due to karyotypic difference stand separate from each other. Therefore karyotypic features may help in differentiating the two species.

T. systola and *T. hoogiana* from the section Tulipanum are placed close to each other and form a group, while *T. micheliana* (sec. Eichler) is joined them with some distance followed by *T. lehmanniana* (sec. Kolpakovskianae). The same relationship is revealed from morphological characters (MATIN 1998). The separation of *T. scherenkii* from the other species is also similar to morphological characters.

PCA analysis of relative karyotypic data revealed that the first 3 factors comprise about 68% of total variance. In the first factor, which comprises about 35% of total variance, the relative length of chromosomes 1, 3 and 5 possessed the highest correlation (>0.60) and are the most variable karyotypic characters of the first factor. This factor separates *T. wilsoniana* from the other species. In the second factor which comprises about 19% of total variance, the relative length of chromosomes 6, 7 and 9 possessed the highest correlation (>0.60). This factor separates the populations of *T. montana* and *T. scherenki* from the other species (Fig. 5).

It is intresting to mention that in Hamedan population of *T. humilis* the first pair of chromosomes was heteromorph and differed in size in all the somatic cells studied (Fig. 2). Various sources have been suggested for the occurrence of asymetrical bivalents including breakdown of a larger complex, defeciencies and duplication heterozygotes, pericentric and paracentric inversion heterozygotes, unequal translocation (SYBENGA 1992) as well as chromosome fragmentation (SHEIDAI *et al.* 2000a). However the reason for the occurrence of asymmeric/ heteromorph chromosomes in *T. humilis* is not known yet.

In short the present study reveals that: 1) *Tulipa* species of Iran possess similar chromosome number ($2n = 24$) and such data is not useful in the species delimitation; 2) the species/ populations vary in their karyotypic formulae and SAT-



Figs. 4 and 5 – UPGMA cluster analysis and PCA ordination of *Tulipa* species. Abbreviations: b = *T. biflora*, h = *T. hoogiana*, l = *T. linifolia*, le = *T. lehmania*, m = *T. micheliana*, mc = *T. montana* var. *crysantha*, mm = *T. montana* var. *mantana*, s = *T. sogdiana*, sch = *T. schrenkii*.

chromosomes and show a low value of r value for short arm of the chromosomes and arm ratio indicating the occurrence of structural changes in the chromosomes; 3) the species studied differ significantly in their total chromatin length, indicating the occurrence of a significant gain/ loss during the species diversification; 4) two varieties of *T. montana* var. *montana* and var. *crysantha* do not differ significantly in their total chromatin length and show inter-population variation in karyotypic details, therefore karyotypic features can not help in differentiating the two varieties from each other and morphological characters are more helpful. However these two varieties are distinct in their karyotypic features compared to *T. linifolia* which are otherwise very similar in morphological characters.This is the first cytlogical report in *Tulipa* species and populations of Iran.

REFERENCES

ATHANASIOU K., 1988 – *Some cytogeographical notes on the genus Tulipa* L. (Liliaceae). Botanika Chronika, 8: 21-59.

BOISSIER E., 1882 – *Flora Orientalis*. Vol V: 191-201. Geneva and Basel.

BOTSCHANTZEVA Z.P., 1982 – *Tulips: Taxonomy, Morhology, Cytology, Phytogeography and Physiology*. English translated edition by H. Q, Varekamp, Rotterdom: Balkema.

HUZIWARA Y., 1962 – *Karyotype analysis in some genera of Compositae. VIII. Further studies on the chromosomes of Aster.* Amer. J. Bot., 49: 116-119.

JOHNSON M.A.T. and BRANDHAM P.E., 1977 – *New chromosome numbers in petaloid monocotyledons and in other miscellaneous angiosperms.* Kew Bulletin, 52: 121-138.

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

MATIN F., 1998 – *Tulips of Iran*. Publication of Agricultural Research, Education and Development. Tehran, Iran.

RAAMSDONK L.W.D. VAN and VARIES T. DE., 1992 – *Biosystematic studies in Tulipa* sec. *Eriostemones (Liliaceae)*. Plant Syst. Evol., 179: 27-41.

RAAMSDONK L.W.D. VAN and VARIES T. DE., 1995 – *Species relationships and taxonomy in Tulipa subg. Tulipa (Liliaceae)*. Plant Syst. Evol., 195: 13-44.

RECHINGER K.H., 1990. – *Tulipa* L. In: K.H. Rechinger (Ed), Flora Iranica, 165: 76-103, Graz.

ROMERO ZARCO C., 1986. – *A new method for estimating karyotype asymmetry*. Taxon, 35: 526-530.

SHEIDAI M., NARENGI Z. and KHATAMSAZ M., 1999 – *Karyotype and seed protein analysis of Lycium(Solanaceae) in Iran.* Edinb. J. Bot., 56: 253-264.

SHEIDAI M., ARMAN M., MOHAMADI-SAEID A. and ZEHZAD B., 2000a – *Notes on cytology and seed protein characteristics of Aegilops species in Iran*. The Nucleus, 43: 118-128.

SHEIDAI M., NASIRZADEH A. and KHERADNAM M., 2000b – *Karyotypic study of Echinops (Asteraceae) in Fars Province, Iran*. Bot. J. Linn. Soc., 134: 453-463.

STEBBINS G.L., 1971 – *Chromosomal evolution in higher plants*. Edwar Arnold Publisher.

SYBENGA J., 1992 – *Cytogenetics in Plant Breeding*. Berlin, Springer-Verlag.

UPCOT M. and LA COUR L., 1936 – *The genetic structure of Tulipa. I. A chromosome survey*. Journal of Genetic, 33: 237-254.

VERMA B.N., 1980 – *Karyotype analysis in three species of Rhizoclonium Kutze*. Cytologia, 45: 433-440.

WENDELBO P., 1977 – *Tulips and Irisis of Iran and their relatives*. Tehran, Botanical Institute of Iran.

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