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Cytotaxonomy of some *Festuca* species and populations in Iran

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Karyotypic study of nine populations belonging to five *Festuca* species showed chromosome numbers of 2n = 2x = 14, 2n = 4x = 28 and 2n = 6x = 42. The chromosome number of *F. elwendiana* is reported for the first time. The chromosomes were mostly metacentric and sub-metacentric. Significant differences in the size of chromosomes and their arm ratios indicated the occurrence of quantitative changes in the chromatin (DNA) material during species diversification. The species also differed in their karyotypic formulae possibly due to the occurrence of chromosome structural changes. The karyotypic data and meiotic characteristics of the species were used for multivariate analysis, which partly supports taxonomic treatment of the genus *Festuca* in the flora of Iran.

Keywords: Cytotaxonomy, *Festuca*, chromosome, karyotype, Iran

Introduction

The genus *Festuca* L. (Poaceae) comprises about 450–500 species distributed in the polar, temperate and alpine regions of both hemispheres. The genus *Festuca* is an ancient group and considered as one of the main evolutionary lines in the tribe Poeae (TZVELEV 1976). *Festuca* species grow wild throughout Iran, varying in number from10–12 according to different authors (PARSA 1950, BOR 1970).

Cytotaxonomical studies have been considered important in showing the phylogenetic relationships of the genus *Festuca* (DUBCOVSKY and MARTINEZ 1992). The wide occurrence of polyploidy and its essential role in the evolution of this group, argue for the importance of cytological studies in the genus *Festuca* (MALIK and THOMAS 1966). The available literature from the other parts of the world dealing with cytogenetic study of *Festuca* (for example: PILS 1980, BULINSKA-RADOMSKA and LESTER 1986, JAUHAR and CRANE 1990, AIKEN and FEDAK 1991, RASKINA et al. 1995, ORTÚÑEZ and DE LA FUNETE 2004) supports such an assumption. However, cytotaxonomical study of the genus *Festuca* is lacking for the species growing wild in Iran.

The present study considers karyotypic analyses of 9 populations of 5 *Festuca* species of Iran with the aim of providing some basic cytogenetic data for the country, and the data

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obtained, along with other cytogenetic information available on chromosome pairing and chiasma frequency of the *Festuca* species, are used to illustrate inter-species relationships.

Materials and methods

Plant material

Karyotypic studies were performed on 9 populations of 5 *Festuca* species, namely 1 – F. *arundinacea* Schreb. (2 populations), 2 – *F. valesiaca* s.l. Schleich. ex Gaudin (4 populations), 3 – *F. gigantea* (L.) Vill. (one population), 4 – *F. elwendiana* Markgr.-Dann. (one population), and 5 – *F. drymeia* Mertens et Koch. (one population). The voucher specimens are deposited in the Herbarium of Shahid Beheshti University (HSB) and TARI. The karyotypic data and the meiotic characteristics of these species along with the meiotic characteristics of *F. akhanii* Tsvelev. were used for phylogenetic analysis.

Cytological studies

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. The fixed tips were then washed thoroughly in distilled water and macerated in 60° C 1N HCL for about 30 seconds. 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 a Camera Lucida and measurements were performed accordingly from such sketches. The chromosomes were identified according to LEVAN et al. (1964), karyotype symmetry was determined according to STEBBINS (1971) and A1 and A2 symmetry indices of ROMERO-ZARCO (1986), while other karyotypic parameters like total form percentage (TF) %, S% (the relative length of shortest chromosome/ total length of chromosomes, HUZIWARA 1962) and coefficient of variation of the chromosome size were also determined (SHEIDAI et al. 2000).

Statistical analyses

The analysis of variance (ANOVA) and the least significant difference test (LSD) were performed to reveal significant differences in the size of chromosomes among the populations of each species as well as among species with similar somatic chromosome numbers (SHEIDAI et al. 2000).

In order to group the species studied based on similarity in their karyotypic features as well as meiotic characteristics, different clustering methods of UPGMA (unweighted paired group with arithmetic average) and WARD (minimum spherical cluster method) as well as ordination based on principal coordinate analysis (PCO) were performed. Since the species studied possess different somatic chromosome numbers, relative karyotypic and meiotic parameters were used in clustering and PCO ordination (Table 3). Only two populations of *F. valesiaca* s.l. possessed both karyotypic and meiotic data and were used as the representative of the species in clustering.

The Euclidean and taxonomic distances were used as dissimilarity coefficient in cluster analysis of cytological data (SHEIDAI et al. 2000). Numerical analyses were performed using SPSS ver. 9 (1998) and NTSYS Ver. 2.2 (1998).

Results

The Evin and Chaloos populations of *F. arundinacea*, the Yaghtehkalan and Sharlogh populations of *F. valesiaca* s.l. as well as the species of *F. gigantea* and *F. elwendiana* possessed 2n = 42 chromosome number, while two populations of *F. valesiaca* s.l. i.e. Heydarbaba mountain and Reineh possessed 2n = 28 chromosome number and the Looh jungle population of *F. drymeia* showed the presence of 2n = 14 (Tabs. 1–3, Figs. 1–4).

The size of chromosomes varied from 18.53 µm in the Yaghtehkalan population of *F. valesiaca* s.l. to 5.04 µm in the Heydarbaba population of *F. valesiaca* s.l. (Tab. 1). The mean chromatin length varied from 8.50 µm in the Chaloos population of *F. arundinacea* to 11.97 µm in the Looh jungle population of *F. drymeia*. Tetraploid populations of *F. valesiaca* s.l. as well as the hexaploid species and populations studied, show low values of mean chromatin length compared to the diploid species of *F. drymeia*. However, no correlation seems to exist between the increase in the chromosome number (ploidy level) and the decrease in the mean chromatin length, as some of the hexaploid populations possess a higher value of the mean chromatin length than the tetraploid species.

The chromosomes were mostly metacentric (m) or sub-metacentric and only in the Evin population of *F. arundinacea* was one sub-telocentric chromosome observed (Tab. 2).

In terms of the STEBBINS two system of karyotype symmetry, the *Festuca* species studied mostly occupy 1A, IB and 2B classes, which are considered rather primitive classes in this system. The species of *F. drymeia* occupy the 1A class and may be considered to pos-

Tab 1. Karyotypic features of *Festuca* species and populations studied.

Species	Locality	2n	L (µm)	S (µm)	TL (µm)	X (µm)	L/S
F. arundinacea1	Tehran, Evin	42	17.78	6.45	224.03	10.66	2.75
F. arundinacea2	Chaloos	42	12.73	5.44	178.59	8.50	2.34
F. gigandea	Golestan, Looh Jungle	42	13.79	5.41	190.41	9.06	2.54
F. valesiaca1	Golestan National Park, Yaghtehkalan	42	18.53	5.82	246.82	11.75	3.18
F. valesiaca2	Sharlogh	42	13.83	5.45	201.63	9.60	2.53
F. valesiaca3	Heydarbaba Mountain	28	12.83	5.04	124.08	8.86	2.54
F. valesiaca4	Damavand, Reineh	28	15.18	6.58	136.56	9.75	2.30
F. elwendiana	Hamedan	42	14.23	5.28	198.88	9.47	2.69
F. drymeia	Golestan, Looh Jungle	14	14.96	8.99	83.82	11.97	1.66

Abbreviations: L = Size of the longest chromosome, S = Size of the shortest chromosome, TL = Total chromatin length, X = Mean chromatin length.

Tab 2. Karyotypic symmetry of *Festuca* species and populations studied.

Species	KF	S%	CV	TF%	ST	A1	A2
F. arundinacea1	18m+2sm+1st	36.27	28.94	38.63	2B	0.28	0.35
F. arundinacea2	17m+4sm	42.73	22.23	39.13	2B	0.22	0.36
F. gigandea	11m+10sm	39.33	25.22	38.08	2B	0.25	0.37
F. valesiaca1	18m+3sm	31.40	28.20	42.65	1B	0.28	0.26
F. valesiaca2	21m	39.40	25.80	42.10	2B	0.25	0.24
F. valesiaca3	11m+3sm	39.28	22.91	41.48	1B	0.30	0.22
F. valesiaca4	12m+2sm	43.30	23.48	40.75	1B	0.31	0.23
F. elwendiana	18m+3sm	37.10	25.02	41.77	2B	0.25	0.29
F. drymeia	7m	60.00	15.95	41.35	IA	0.15	0.30

Abbreviations: KF = Karyotypic formulae, S% = The relative length of shortest chromosome, CV = coefficient of variation of the chromosomes size, ST = Stebbins class, A1 = Inter-chromosomal symmetry index, A2 = Intra-chromosomal symmetry index.

sess the most symmetrical (primitive) karyotype. This species also showed the lowest value of coefficient of variation among the species studied. Three populations of *F. valesiaca* s.l. occupy the 1A class while the Sharlogh population of *F. valesiaca* s.l. along with populations of *F. arundinacea* and *F. gigantea* occupy the 2B class of the STEBBINS system.

By using the ROMERO-ZARC symmetry indices of A_1 and A_2 we can determine the more asymmetric karyotype among the species having similar STEBBINS classes of symmetry. For example among the species with the 1B class, the Reineh population of *F.* valesiaca s.l. possesses the highest A1 value (0.31) and therefore a more asymmetric karyotype. Similarly, among the species with the 2B symmetry class, *F. gigantea* possessed the highest value for A_1 (0.37) and the highest asymmetric karyotype.

Tab 3. Relative cytological characters used in cluster analysis.

Species	A2	A1	S%	CV	TF%	X	L/S	RBN	RDN	IN	IVN	IXN	TXN	TOXN
F. arundinacea1	0.28	0.35	36.27	28.94	38.63	10.66	2.75	0.90	0.08	0.03	0.00	0.36	1.54	1.90
F. arundinacea2	0.22	0.36	42.73	22.23	39.13	8.50	0.34	0.79	0.19	0.02	0.00	0.37	1.05	2.00
F. gigantea	0.25	0.37	39.33	25.22	38.08	9.06	2.54	–	–	–	–	–	–	–
F. valesiaca1	0.28	0.26	31.40	28.20	42.65	11.75	3.18	0.75	0.21	0.01	0.02	0.04	1.74	1.77
F. valesiaca2	0.25	0.24	39.40	25.80	42.10	9.60	2.53	0.42	0.55	0.01	0.01	0.01	1.43	1.44
F. elwendiana	0.25	0.29	37.10	25.02	41.77	9.47	2.69	–	–	–	–	–	–	–
F. drymeia	0.15	0.30	60.00	15.95	41.35	11.97	1.66	0.80	0.20	0.00	0.00	0.49	1.15	1.64
F. akhanii	–	–	–	–	–	–	–	0.77	0.21	0.00	0.01	0.18	1.64	1.82

Abbreviations: A2 = Inter-chromosomal symmetry index, A1 = Intra-chromosomal symmetry index, S% = Relative length of the shortest chromosome, CV = coefficient of variation of the chromosomes size, TF%= Total form percentage, X = Mean chromatin length, L/S = longest/shortest chromosome ratio, RBN = Number of ring bivalents/cell, RDN = Number of ROD bivalents /cell, IN = Number of univalents/ cell, IVN = Number of quadrivalents/cell, IXN = Number of intercalary chiasmata/bivalent, TXN = Number of terminal chiasmata/bivalent, TOXN = Number of total chiasmata/bivalent.

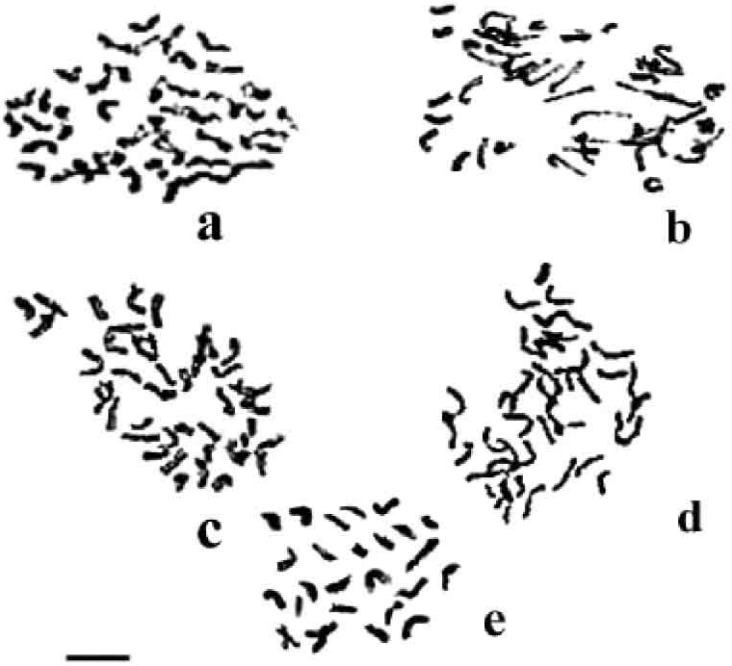

Fig. 1. Representative mitotic metaphase cells in *Festuca* species studied. *F. arundinacea*, Evin population (2n = 42) (a); *F. arundinacea*, Chaloos population (2n = 42) (b); *F. elwendiana* (2n = 42) (c); *F. valesiaca* s.l., Sharlogh population (2n = 42) (d); *F. valesiaca* s.l., Heydarbaba population (2n = 28) (e). Scale bar denotes 15 μm

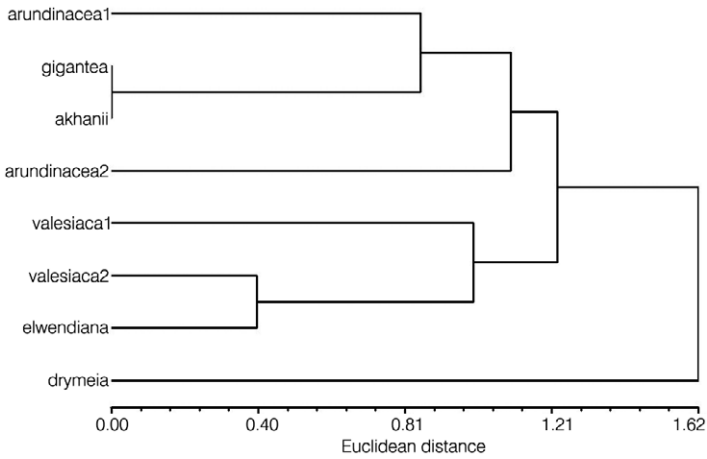

Fig. 2. UPGMA clustering of the *Festuca* species studied.

Groupings of the species are studied based on their relative karyotypic as well as meiotic characteristics (Tab. 3, Figs. 3–4). Different clustering methods like UPGMA, WARD produced similar results from which UPGMA possessed the highest cophenetic correlation

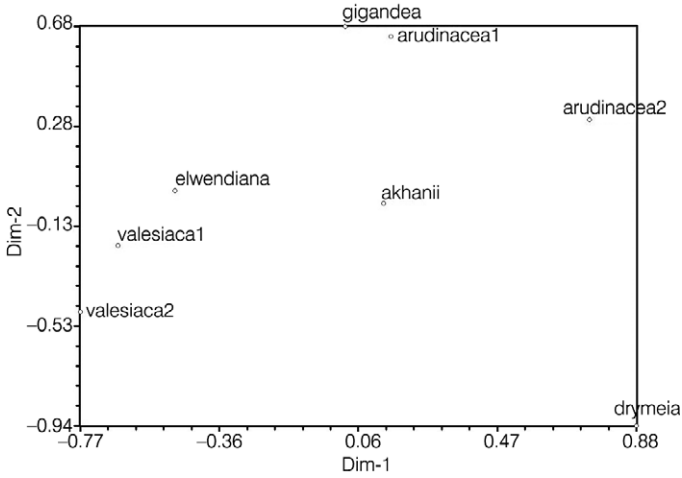

Fig. 3. PCO plot of the *Festuca* species studied.

(r = 0.71). In both analyses three major clusters are observed. The first major cluster is composed of *F. arundinacea*, *F. gigantea* and *F. akhanii* while, the species of *F. valesiaca* s.l. and *F. elwendiana* form the second major cluster. *F. drymeia* stands alone in the third cluster. PCO ordination partly supports the clustering result as *F. akhanii* stands far from the species of *F. arundinacea*, *F. gigantea* in a position between the two major clusters.

Discussion

The base chromosome number in the genus *Festuca* is x = 7 (CLAYTON and RENOVIZE 1986), therefore the species of *F. arundinacea*, *F. elwendiana*, *F. gigantea* are hexaploid (6x), while *F. drymeia* is diploid (2x). The tetraploid (4x) and hexaploid chromosome number probably characterized *F. valesiaca* s.l. populations.

The chromosome numbers of *F. arundinacea*, *F. gigantea*, *F. valesiaca* s.l. and *F. drymeia* supported previous studies (MALIK and THOMAS 1966, MIZIANTY and PAWLUS 1982, BULINSKA-RADOMSKA and LESTER 1986, STEPANOV and MURATOVA 1995), while the chromosome number of *F. elwendiana* is reported for the first time.

Polyploidy is considered one of the important mechanisms in the evolution of grass species including *Festuca*. Different populations of several *Festuca* species show numerical chromosome polymorphism, for example MIZIANTY and PAWLUS (1982) reported a diploid (2n = 14) and a hexaploid (2n = 42) chromosome number for *F. valesiaca* s.l., while KOZUHAROV and PETROVA (1991) reported diploid (2n = 14) and tetraploid levels (2n = 28) for *F. valesiaca* var. *valesiaca*. The present study also shows the occurrence of 2n = 4x = 28 and 2n = 6x = 42 for different populations of *F. valesiaca* s.l. Different polyploidy levels have also been reported for *F. arundinacea* ranging from 2n = 4x = 28 to 2n = 10x = 70 (BULINSKA-RADOMSKA and LESTER 1986). According to LEVITSKY and KUZMINA (1927) some of these polyploids correspond to the varieties recognized by SAINT-YVES (1927). Again, the present study reports 2n = 6x = 42 for *F. gigantea* while STRID and FRANZEN (1981) have reported 2n = 4x = 28 for the same species. Therefore the present study as well

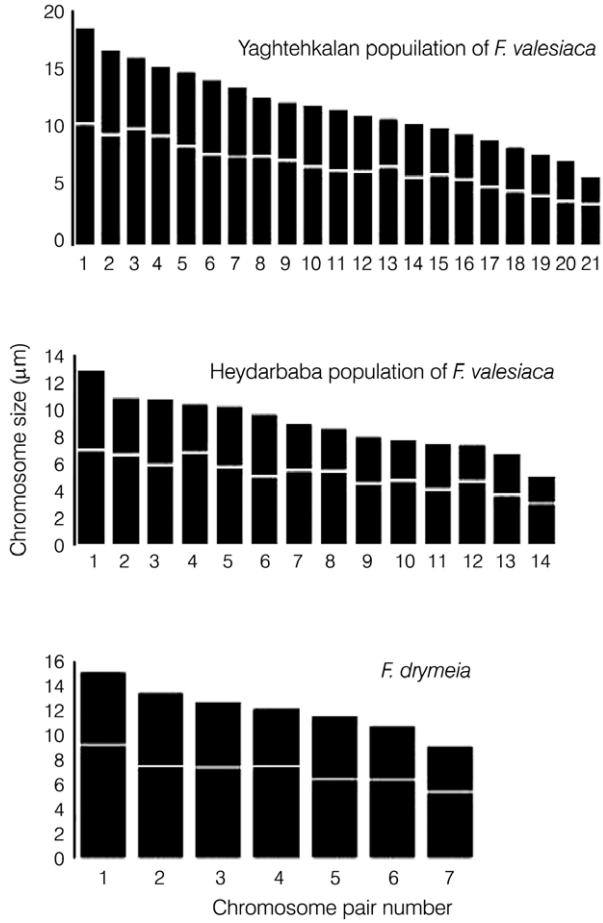

Fig. 4. Representative ideograms of *Festuca* species studied. (Scale bars are in micrometers).

as the earlier cytological reports on the species and populations of *Festuca* show the changes in the chromosome number as a main mechanism of species diversification in the genus *Festuca*.

ANOVA and LSD tests showed the presence of significant difference ($p < 0.05$) in the size of chromosomes as well as the ratio of long arms to short arms among tetraploid and hexaploid populations and species, indicating a significant quantitative change in the amount of chromatin (DNA) in the *Festuca* species diversification.

Difference in the karyotypic formulae of the species and populations studied may indicate the occurrence of chromosomes structural changes like translocations as also evidenced by quadrivalent formation in metaphase of meiosis-I in some populations of *F. arundinacea*. The allohexaploid species with diplontic behavior could characterize *F. valesiaca* s.l. (unpublished).

The coefficient of variation of chromosome size in the karyotype shows the amount of variation in the size of chromosomes in a species, therefore the higher coefficient of varia-

tion obtained for the karyotype in the Evin population of *F. arundinacea* than that of the Chaloos population and also the higher coefficient of variation of the size of chromosomes in the Yaghtehkalan population of *F. valesiaca* s.l. than that of the Sharlogh population may also support the occurrence of chromosome structural changes in these species.

Considering the coefficient of variation changes of the chromosomes size among diploid, tetraploid and hexaploid species studied, the lowest value occurs in the diploid species of *F. drymeia* (15.95), tetraploid populations of *F. valesiaca* s.l. show a bit higher coefficient of variations of the chromosomes size, followed by the hexaploid populations and species (except Chaloos population of *F. arundinacea*). Therefore it seems that in general the variation in the size of chromosomes increases with an increase in the chromosome number. This conclusion needs to be confirmed by studying a higher number of *Festuca* species wtih different chromosome numbers.

The species of *F. elwendiana, F. valesiaca* and *F. akhanii* are among the *Festuca* species that have filiform leaves and are considered close to each other in Flora Iranica (BOR 1970), while the species of *F. gigantea*, *F. arundinacea* and *F. drymeia* are among the *Festuca* species that have flat leaves, and are also considered to be close to each other in Flora Iranica (BOR 1970). Our phylogenetic analysis of the same species based on the morphological characters (unpublished data) supports the taxonomic treatment of the Flora Iranica (BOR 1970).

However, the grouping of the *Festuca* species studied based on cytological data used, partly agrees with the taxonomic treatment of the genus *Festuca* in the Flora Iranica (BOR 1970) and phylogenetic analysis of the same species based on morphological characters, as *F. drymeia* stands far from *F. gigantea* and *F. arundinacea* and *F. akhanii* stands far from *F. elwendiana* and *F. valesiaca*. This may be due to some missing data available in cytological analysis or different evolutionary history of cytological features and morphological characters in the species studied.

In general cytological studies of the *Festuca* species growing wild in Iran indicate the role of polyploidy, chromosome structural changes and quantitative change in the amount of DNA in species diversification and suggests that such data may be used in the taxonomy and phylogenetic consideration of the genus.

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