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COMPARATIVE CYTOGENETIC STUDY OF SOME GRASS GENERA OF THE SUBFAMILY POOIDEAE IN IRAN

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Abstract. The cytogenetic characteristics of 53 grass species belonging to 8 genera of subfamily Pooideae were compared in terms of ploidy level, chromosome pairing, heterozygote translocation, unreduced gamete formation and B chromosomes. The genera studied possessed species with diploid, tetraploid and hexaploid chromosome numbers; the genus *Melica* was an exception with its very homogenous group of mainly diploid species. In the genera *Aegilops*, *Bromus*, *Stipa* and *Avena*, some tetraploid and hexaploid species showed diplontic behavior, possibly due to their allopolyploid nature or to mechanisms controlling chromosome pairing, while some diploid and allopolyploid species such as *Bromus brachystachys*, *Festuca arundinacea* and *Secale cereale* subsp. *cereale* formed quadrivalents due to heterozygote translocations. The studied genera differed significantly in their chiasma frequency and distribution as well as chromosome pairing, indicating their genetic distinctness. Unreduced gametes were formed in some of the species due to cytomixis or anaphase failure.

Key words: Cytogenetic analysis, chromosome, heterozygote translocation, Pooideae, polyploidy

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INTRODUCTION

The grass family (Poaceae) is the fourth-largest flowering plant family, with 651 genera and about 10,000 species (Clayton & Renvoize 1986; Gaut 2002). Grasses are found throughout the globe and can dominate temperate and tropical habitats. Altogether, grasses cover >20% of the earth's land surface (Shantz 1954), and are a major food source for humans. Three-grain crops – wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.) and maize (*Zea mays* L.) – are predominant food sources, but the grasses also include several other and perhaps under-appreciated crops. For example, turfgrasses (*Lolium* L., *Festuca* L.) are a major crop group. The economic incentive to work on the grasses is substantial, and their ecological dominance makes them intriguing from an evolutionary viewpoint. As a result, grasses have been the subject of intense phylogenetic, ecological, agronomic and molecular study.

Grasses have been grouped into two major clades, BOP (Bambusoideae, Oryzoideae, Pooideae) and PACC (Panicoideae, Arundinoideae, Chloridoideae + Centothecoideae) by the Grass Phylogeny Working Group (Kellogg 2000, 2001). The latter two subfamilies of BOP include the economically important species rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and oat (*Avena sativa* L.).

Fossil evidence indicates that Poaceae may have first appeared in the Late Cretaceous, approximately 70 million years ago. Although there are many fossil records for the grass family, the ambiguity caused by their similarity to several related families such as Cyperaceae and Juncaceae greatly reduces their utility. Molecular studies suggest that the grass family originated roughly 77 million years ago. The divergence between Oryzoideae (rice) and the Pooideae (oat, barley and wheat) is estimated at 46 my, and this represents the time of origin of the BEP clade (Bambusoideae, Oryzoideae + Pooideae); within the Pooideae, the Triticeae (barley and wheat) diverged from oats *ca* 25 my; barley and wheat diverged *ca* 13 my ago, an estimate similar to a previous one of 10 my (Wolfe *et al.* 1989).

Our earlier reports on the cytogenetics of

Gramineae species give some basic data about the chromosome numbers, chiasma frequency and ploidy levels in some of the species studied here (Sheidai *et al*. 1999, 2002, 2003; Sheidai & Fedaei 2005; Sheidai & Begheri-Shabestarei 2007). The present paper reports a comparative cytogenetic analysis of species belonging to eight genera (*Aegilops*, *Avena*, *Bromus*, *Festuca*, *Melica*, *Milium*, *Secale*, *Stipa*; Pooideae) growing in Iran, considered in terms of ploidy level, chromosome pairing, chiasma frequency and distribution, the occurrence of heterozygote translocations, diplontic behavior and B chromosomes.

Most studies have supported the monophyly of the Pooideae, with one exception (Cummings *et al*. 1994). This result indicates that Pooideae is a group of very closely related taxa and is in conformity with its obvious diagnostic morphological characters of the C3 pathway, unique stoma, and the absence of microhairs throughout the subfamily (Ellis 1987). There are some problematic tribes or genera, however, such as *Aristedeae*, *Stipeae*, *Bromus* and *Ehrharta*. These tribes or genera often share morphological and anatomical characters from different groups, and their taxonomic positions are not well resolved.

Material and methods

PLANT MATERIAL

For cytogenetic study, plants were collected in Iran from natural habitats of the following 53 species/taxa of the eight genera:

Aegilops L. – *A. crassa* Boiss., *A. cylindrica* Host, *A. speltoides* Tausch, *A. tauschii* Coss., *A. triuncialis* L., and *A. umbellulata* Zhuk.

Avena L. – *A. barbata* Pott *ex* Link, *A. eriantha* Durieu, *A. fatua* L., *A. sterilis* subsp. *ludoviciana* (Durieu) M. Gillet & Magne, and *A. wiestii* Steud.

Bromus L. sect. *Bromus* – *B. brachystachys* Hornung., *B. briziformis* Fisch. & C. A. Mey., *B. japonicus* Thumb. var. *japonicus*, *B. lanceolatus* Roth var. *lanceolatus*, *B. rechingeri* Melderis, *B. scoparius* L. var. *scoparius*, and *B. squarrosus* L., and *Bromus* sect. *Genea* Dumort. – *B. fasciculatus* Presl., *B. madritensis* L., *B. rubens* L., *B. sericeus* Drobow, *B. sterilis* L., and *B. tectorum* L.

Festuca L. sect. *Festuca* – *F. arundinacea* Schreb., *F. valesiaca* Schleich. *ex* Gaudin, *F. akhanii* Tzvelev, and *F. drymeja* Mert. & Koch.

Melica L. – *M. persica* Kunth subsp. *persica*, *M. persica* subsp. *inaequiglumis* (Boiss.) Bor, *M. jacquemontii* Decne. subsp. *jacquemontii*, *M. jacquemontii* subsp. *canescens* (Regel) Bor, *M. jacquemontii* subsp. *hohenackeri* (Boiss.) Bor, *M. ciliata* L. var. *ciliata*, and *M. picta* K. Koch.

Milium L. – *M. vernale* M. Bieb., and *M. schmidtianum* K. Koch.

Secale L. – *Secale cereale* L. subsp. *cereale*, *S. cereale* subsp. *ancestrale* Zhuk., *S. strictum* C. Presl subsp. *strictum*;

Stipa L. sect. *Stipa* – *S. caucasica* Schmalh, *S. lessingiana* Trin. & Rupr., *S. pennata* L., and *S. turkestanica* Hackel; *Stipa* sect. *Barbatae* Junge *emend*. Freitag. – *S. arabica* Trin. & Rupr., *S. ehrenbergiana* Trin. & Rupr., *S. hohenackeriana* Trin. & Rupr., *S. holosericea* Trin., and *S. iranica* Freitag; *Stipa* sect. *Lasiagrostis* (Link) Hackel – *S. caragana* Trin., and *S. haussknechtii* Boiss.; *Stipa* sect. *Stipella* Tzvelev *emend*. Freitag. – *S. capensis* Thunb., and *S. parviflora* Desf.

The list of localities is provided in the Appendix. Voucher specimens are deposited in the herbarium of Shahid Beheshti University (HSBU) and the Research Institute of Forests and Rangelands (TARI) in Tehran.

CYTOLOGICAL PREPARATION

For cytogenetic study, fifty flower buds from ten randomly selected plants were used. The squash technique and pollen fertility test followed protocols described earlier (Sheidai *et al*. 2005).

Different methods were used to detect 2n gametes, including morphological, flow cytometry and cytological methods. The most direct method of screening for 2n pollen involves examination of the size range of pollen produced by an individual: cell volume increases with the increase in DNA content, and this in turn affects pollen diameter (Villeux 1985). Taking advantage of this regularity, normal and potential unreduced (2n) pollen grains were sketched and measured from camera lucida images.

Relative cytogenetic data from species and genera having different chromosome numbers were used to compare cytogenetic characteristics such as chiasma frequency and distribution, as well as chromosome association. For this purpose the data were divided by the number of chromosomes so that the same data per bivalent were obtained. The same was done for bivalents, quadrivalents, etc., to obtain data per cell.

In order to determine the significance of interspecific differences between the cytogenetic characteristics

studied, analysis of variance (ANOVA) followed by the least significant difference test (LSD) was performed for the genotypes using the relative data. Statistical analyses used SPSS ver. 9 software.

Results and discussion

Polyploidy and chromosome pairing

Details of cytogenetic characters are presented in Tables 1–5 and Fig. 1. This section addresses the basic chromosome number, ploidy level, chromosome pairing, and the occurrence of heterozygote translocation in the grass genera studied.

The species of *Aegilops* studied possessed 2n = 2x = 14, 2n = 4x = 28 and 2n = 6x = 42 chromosome numbers, with *A. crassa* having both tetraploid and hexaploid populations. Tetraploid species showed diplontic behavior and formed bivalents only, while diploid species of *A. speltoides* showed the occurrence of quadrivalents and hexavalents, possibly due to its outbreeding and structural heterozygosity.

Diploid-like behavior has been reported in polyploid *Aegilops* species, in spite of the presence of several structurally and genetically similar chromosome sets which could pair both homologously and homoeologously. Such diplontic behavior is suggested to be due to the existence of a diplodizing genetic system (McGuire & Dvorák 1982). *A. triuncialis* possessed the highest value of relative total chiasmata (1.93) and terminal chiasmata (1.69), while the diploid population of *A. crassa* had the highest value for intercalary chiasmata (0.53) (Table 2).

The *Avena* species studied possessed 2n = 2x = 14, 4x = 28 and 6x = 42 chromosome numbers; they formed only bivalents in metaphase of meiosis I, showing diplontic behavior and no chromosome structural heterozygosity. *Avena eriantha* possessed both diploid and tetraploid chromosome numbers.

Among the *Avena* species studied, the highest value of relative total (2.14) and terminal (1.77) chiasmata occurred in the tetraploid population of *Avena eriantha*, while the value of intercalary chiasmata was highest (0.47) in diploid and tetraploid populations of *A. eriantha* (Table 2).

The 2n chromosome numbers of the studied *Bromus* species were 2n = 2x =14 or 2n = 4x = 28 (Table 1). *Bromus sericeous* is Iran's only *Bromus* species possessing diploid and tetraploid chromosome numbers. The values of relative intercalary (1.12) and total (2.90) chiasmata were highest in *B. squarrosus* (Table 2). Among the diploid species studied, only *B. brachystachys* showed the occurrence of quadrivalents, possibly due to heterozygote translocation. Tetraploid species of *B. lanceolatus* var. *lanceolatus*, *B. rechingeri*, *B. sericeous* and *B. tectorum* showed diplontic behavior and formed only bivalents in metaphase of meiosis I, possibly due to the allopolyploid nature of these species or to the mechanisms controlling chromosome pairing (Stebbins 1981; Armstrong 1991; Ainouche *et al*. 1995, 1999).

The species of *Festuca* studied possessed 2n = 2x = 14 and 2n = 6x = 42 chromosome numbers. *Festuca arundinacea* (tall fescue, AABBCC) is known to form only bivalents, due to the presence of a genetic regulation system that ensures bivalent formation by suppressing homoeologous chromosome pairing and disomic inheritance (Jauhar 1975; Jauhar & Crane 1990). Such a controlling mechanism is believed to operate in other natural polyploid species of *Festuca* as well (Jauhar 1975). The present study shows that *F. arundinacea* does not exhibit diplontic behavior, and forms quadrivalents in metaphase of meiosis I, possibly due to the occurrence of heterozygote translocation (Table 1). The situation is similar in *F. valesiaca* and *F. akhanii*.

The studied species of *Melica* were all diploid, with 2n = 2x = 18 chromosome number, forming only bivalents in metaphase of meiosis I. In this work no structural heterozygosity was observed in these species, although polyploidy and interspecific hybridization is considered to be highly important in the evolution of Gramineae (Stebbins 1982, 1985). The genus *Melica*, exceptionally, comprises a very homogenous group of mainly diploid species, rarely has a different basic chromosome number, and shows a very low level of interspecific hybridization (Mejia-Saulés & Bisby 2000).

The *Milium* species studied are all diploids with 2n = 2x = 14 and 18 chromosome numbers,

Table 1. Cytogenetic characters studied. n – haploid chromosome number, RB – mean number of ring bivalents, RD – mean number of rod bivalents, I – mean number of univalents, IV – mean number of hexavalents, IX – mean number of intercalary chiasmata, TX – mean number of terminal chiasmata, TOX – mean number of total chiasmata.

Species	n	RB	RD	I	IV	IX	TX	TOX
Aegilops crassa	14	9.80	3.52	0.21	0.00	7.35	16.35	23.70
A. crassa	21	14.25	6.15	0.00	0.00	3.40	31.90	35.30
A. cylindrica	14	11.44	2.41	0.41	0.00	2.48	23.55	26.03
A. speltoides	7	5.20	0.85	0.30	0.03	2.10	10.50	12.15
A. tauschii	7	4.40	2.25	0.35	0.00	2.40	7.09	10.30
A. triuncialis	14	11.43	2.56	0.00	0.00	3.37	23.68	27.06
A. umbelulata	7	6.00	1.00	0.00	0.00	1.46	11.58	13.14
Avena barbata	14	10.55	3.23	0.26	0.00	3.94	21.61	25.55
A. eriantha	7	5.18	1.64	0.36	0.00	3.27	8.73	12.00
A. eriantha	14	13.46	0.54	0.00	0.00	5.09	24.81	29.90
A. fatua	21	16.08	4.69	0.46	0.00	8.69	30.31	39.00
A. sterilis subsp. *ludviciana*	21	17.37	3.26	0.74	0.00	7.16	32.26	39.42
A. wiestii	14	9.86	4.14	0.00	0.00	6.43	21.29	27.71
Bromus brachystachys	7	6.58	0.27	0.00	0.02	1.65	13.35	15.00
B. briziformis	7	6.03	0.97	0.00	0.00	4.06	11.80	15.86
B. fasciculatus	14	10.50	2.33	0.95	0.01	4.95	20.16	25.11
B. japonicus var. *japonicus*	7	4.18	2.82	0.00	0.00	2.71	10.53	13.24
B. lanceolatus var. *lanceolatus*	14	10.83	3.18	0.00	0.00	9.30	24.31	33.61
B. madritensis	14	12.55	1.22	0.22	0.00	5.88	24.12	30.00
B. rechingeri	14	10.92	2.98	0.00	0.00	6.33	22.17	28.50
B. rubens	14	11.66	2.06	0.27	0.01	2.63	23.27	25.90
B. scoparius var. *scoparius*	7	5.18	1.82	0.00	0.00	0.75	12.36	13.11
B. sericeous	7	4.60	0.60	1.80	0.00	1.80	8.80	10.60
B. sericeous	14	12.83	0.83	0.33	0.00	7.16	24.33	31.49
B. squarrosus	7	6.41	0.61	0.00	0.00	7.81	12.49	20.30
B. sterilis	7	5.56	1.28	0.12	0.00	2.28	11.44	13.72
B. tectorum	7	5.67	1.14	0.14	0.00	2.29	10.96	13.25
Festuca akhanii	21	16.20	4.51	0.10	0.01	3.84	34.34	38.18
F. arundinacea	21	18.96	1.62	0.55	0.00	7.60	32.32	39.92
F. drymeja	7	5.59	1.41	0.00	0.00	3.41	8.06	11.47
F. valesiaca	21	15.66	4.44	0.31	0.02	0.76	36.50	37.26
Melica ciliata var. *ciliata*	9	8.13	0.85	0.04	0.00	0.29	16.40	16.69
M. jacquemontii subsp. *canescens*	9	8.85	0.15	0.00	0.00	0.15	17.23	17.38
M. jacquemontii subsp. *hohenackeri*	9	8.47	0.50	0.06	0.00	0.52	16.39	16.88
M. jacquemontii subsp. *jacquemontii*	9	5.88	3.11	0.02	0.00	0.32	11.29	11.62
M. persica subsp. *inaequiglumis*	9	7.60	1.40	0.00	0.00	0.37	15.87	16.23
M. persica subsp.*persica*	9	6.87	2.13	0.00	0.00	0.27	15.33	15.63
M. picta	9	7.96	1.02	0.04	0.00	0.25	16.42	16.67
Milium schmidtiaum	7	6.38	0.60	0.00	0.00	0.36	16.73	17.09
M. vernale	7	6.19	0.80	0.02	0.00	0.22	12.92	13.14
M. vernale	9	9.00	0.00	0.00	0.00	0.33	17.33	17.67
Secale cereale subsp. *ancestrale*	7	5.70	1.10	0.10	0.00	0.20	11.20	12.80
S. cereale subsp. *cereale*	7	6.20	0.40	0.20	0.00	2.40	11.60	14.00
S. strictum subsp. *strictum*	7	5.80	4.00	0.30	0.14	1.80	11.90	13.70
Stipa arabica	22	15.26	4.41	0.52	0.00	1.97	36.82	38.79
S. capensis	18	12.75	5.25	0.00	0.00	0.55	30.20	30.75
S. caragana	12	7.25	4.68	0.14	0.00	1.46	18.64	20.10
S. caucasica	22	9.30	6.19	0.76	0.00	6.03	27.19	33.22
S. ehrenbergiana	22	16.60	1.66	0.33	0.06	3.56	37.30	40.86
S. haussknechtii	22	13.50	8.00	0.20	0.01	1.10	34.47	35.60
S. hohenackeriana	22	17.85	2.25	0.37	0.00	1.59	38.59	40.18
S. holosericea	22	14.33	2.55	0.33	0.05	4.94	34.88	39.83
S. iranica	22	14.14	2.85	0.51	0.04	4.66	32.88	37.44
S. lessingiana	22	12.63	3.43	0.06	0.00	5.80	30.80	36.60
S. parviflora	22	7.76	13.39	0.05	0.02	3.46	28.66	32.12
S. pennata	22	8.69	13.15	0.00	0.00	1.11	29.81	30.92
S. turkestanica	22	14.64	1.76	0.00	0.00	5.48	34.48	39.96

Table 2. Relative cytogenetic characters studied. RBN – mean number of ring bivalents/cell, RDN – mean number of rod bivalents/cell, IN – mean number of univalents/cell, IVN – mean number of hexavalents/cell, IXN – mean number of intercalary chiasmata/bivalent, TXN – mean number of terminal chiasmata/bivalent, TOXN – mean number of total chiasmata/bivalent.

Species	RBN	RDN	IN	IVN	IXN	TXN	TOXN
Aegilops crassa (4x)	0.70	0.25	0.02	0.00	0.53	1.17	1.69
A. crassa (*6x*)	0.68	0.29	0.00	0.00	0.16	1.52	1.68
A. cylindrica	0.82	0.17	0.03	0.00	0.18	1.68	1.86
A. speltoides	0.74	0.12	0.04	0.00	0.30	1.50	1.74
A. tauschii	0.63	0.32	0.05	0.00	0.34	1.01	1.47
A. triuncialis	0.82	0.18	0.00	0.00	0.24	1.69	1.93
A. umbelulata	0.86	0.14	0.00	0.00	0.21	1.65	1.88
Avena barbata	0.75	0.23	0.02	0.00	0.28	1.54	1.83
A. eriantha (2x)	0.74	0.23	0.05	0.00	0.47	1.25	1.71
A. eriantha (4x)	0.96	0.04	0.00	0.00	0.36	1.77	2.14
A. fatua	0.77	0.22	0.02	0.00	0.41	1.44	1.86
A. sterilis subsp. *ludviciana*	0.83	0.16	0.04	0.00	0.34	1.54	1.88
A. wiestii	0.70	0.30	0.00	0.00	0.46	1.52	1.98
Bromus brachystachys	0.94	0.04	0.00	0.00	0.24	1.91	2.14
B. briziformis	0.86	0.14	0.00	0.00	0.58	1.69	2.27
B. fasciculatus	0.75	0.17	0.07	0.00	0.35	1.44	1.79
B. japonicus var. *japonicus*	0.60	0.40	0.00	0.00	0.39	1.50	1.89
B. lanceolatus var. *lanceolatus*	0.77	0.23	0.00	0.00	0.66	1.74	2.40
B. madritensis	0.90	0.09	0.02	0.00	0.42	1.72	2.14
B. rechingeri	0.78	0.21	0.00	0.00	0.45	1.58	2.04
B. rubens	0.83	0.15	0.02	0.00	0.19	1.66	1.85
B. scoparius var. *scoparius*	0.74	0.26	0.00	0.00	0.11	1.77	1.87
B. sericeous (2x)	0.92	0.06	0.02	0.00	0.51	1.74	2.25
B. sericeous (4x)	0.66	0.09	0.26	0.00	0.26	1.26	1.51
B. squarrosus	0.92	0.09	0.00	0.00	1.12	1.78	2.90
B. sterilis	0.79	0.18	0.02	0.00	0.33	1.63	1.96
B. tectorum	0.81	0.16	0.02	0.00	0.33	1.57	1.89
Festuca akhanii	0.77	0.21	0.00	0.00	0.18	1.64	1.82
F. arundinacea	0.90	0.08	0.03	0.00	0.36	1.54	1.90
F. drymeja	0.80	0.20	0.00	0.00	0.49	1.15	1.64
F. valesiaca	0.75	0.21	0.01	0.00	0.04	1.74	1.77
Melica ciliata var. *ciliata*	0.90	0.09	0.00	0.00	0.03	1.82	1.85
M. jacquemontii subsp. *canescens*	0.98	0.02	0.00	0.00	0.02	1.91	1.93
M. jacquemontii subsp. *hohenackeri*	0.94	0.06	0.01	0.00	0.06	1.82	1.88
M. jacquemontii subsp. *jacquemontii*	0.65	0.35	0.00	0.00	0.04	1.25	1.29
M. persica subsp. *inaequiglumis*	0.84	0.16	0.00	0.00	0.04	1.76	1.80
M. persica subsp.*persica*	0.76	0.24	0.00	0.00	0.03	1.70	1.74
M. picta	0.88	0.11	0.00	0.00	0.03	1.82	1.85
Milium schmidtiaum	0.91	0.09	0.00	0.00	0.05	2.39	2.44
M. vernale ($x = 7$)	0.88	0.11	0.00	0.00	0.03	1.85	1.88
M. vernale ($x = 9$)	1.00	0.00	0.00	0.00	0.04	1.93	1.96
Secale cereale subsp. *ancestrale*	0.81	0.16	0.01	0.00	0.03	1.60	1.83
S. cereale subsp. *cereale*	0.89	0.06	0.03	0.00	0.34	1.66	2.00
S. strictum subsp. *strictum*	0.83	0.57	0.04	0.02	0.26	1.70	1.96
Stipa arabica	0.69	0.20	0.02	0.00	0.09	1.67	1.76
S. capensis	0.71	0.29	0.00	0.00	0.03	1.68	1.71
S. caragana	0.60	0.39	0.01	0.00	0.12	1.55	1.68
S. caucasica	0.42	0.28	0.03	0.00	0.27	1.24	1.51
S. ehrenbergiana	0.75	0.08	0.02	0.00	0.16	1.70	1.86
S. haussknechtii	0.61	0.36	0.01	0.00	0.05	1.57	1.62
S. hohenackeriana	0.81	0.10	0.02	0.00	0.07	1.75	1.83
S. holosericea	0.65	0.12	0.02	0.00	0.22	1.58	1.81
S. iranica	0.64	0.13	0.02	0.00	0.21	1.49	1.70
S. lessingiana	0.57	0.16	0.00	0.00	0.26	1.40	1.66
S. parviflora	0.35	0.61	0.00	0.00	0.16	1.30	1.46
S. pennata	0.40	0.60	0.00	0.00	0.05	1.36	1.41
S. turkestanica	0.67	0.08	0.00	0.00	0.25	1.57	1.82

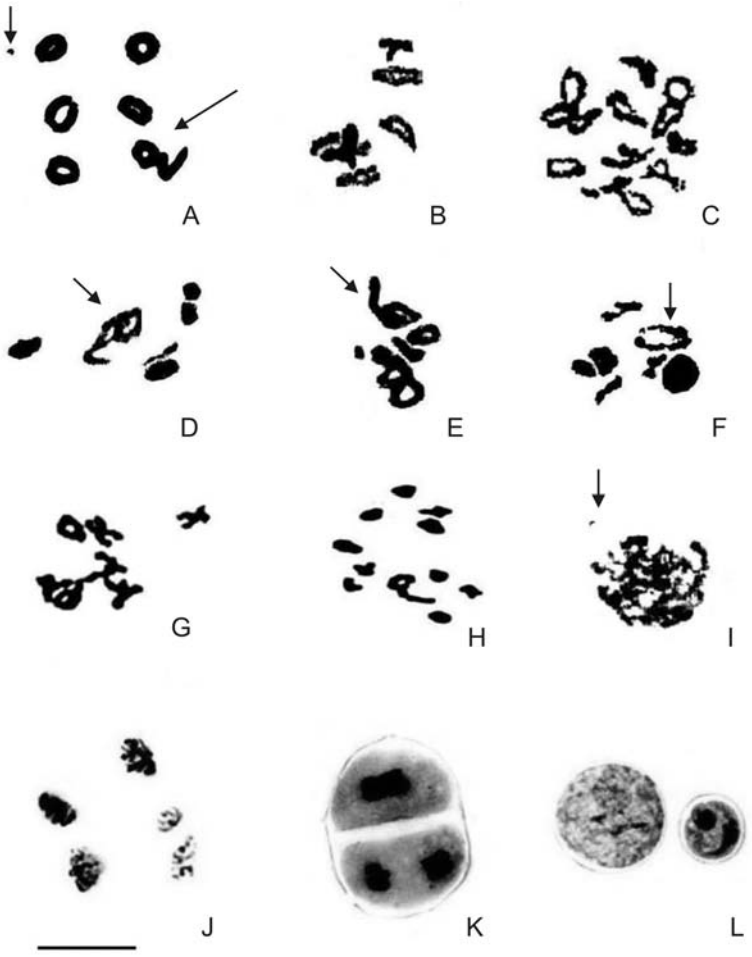

Fig. 1. Representative meiotic cells of the grass species studied. A – meiotic cell of *Bromus rechingeri* showing a quadrivalent (bigger arrow) and single B chromosome (smaller arrow); B & C – meiocytes of *Avena eriantha* showing n = 7 and n = 14 respectively; D & E – meiocytes of *Aegilops speltoides* showing a quadrivalent (arrow); F – meiocyte of *Milium schmidtianum* showing a quadrivalent (arrow); G – normal meiocyte of *Festuca drymeja* showing n = 7; H – aneuploid meiocyte of *Festuca drymeja* showing n = 12; I – B chromosome (arrow) of *Avena barbata*; J – multipolar cell of *Bromus lanceolatus* var. *lanceolatus*; K – meiocyte showing anaphase II failure in *Bromus briziformis*; L – unreduced pollen grain (bigger pollen) of *Bromus japonicus* var. *japonicus*. Scale bar = 10 μm.

showing two different basic chromosome numbers of x = 7 and x = 9. In *Milium*, different basic numbers of x = 4, 5, 7, and 9 have been reported, and diploid, tetraploid and hexaploid levels found (Clayton & Renovize 1986; Tzvelev 1976, 1984; Simon & Tomas 1991; Watson & Dallwitz 1992). *Milium vernale* showed two different diploid chromosome numbers of 2n = 14 and 18, indicating the role of polyploidy in the population divergence of this species. *Milium schmidtiaum* showed the formation of quadrivalents due to heterozygote translocation.

The three subspecies of *Secale* studied possessed 2n = 2x = 14 chromosome number (Table 1). *Secale strictum* subsp. *strictum* was the only subspecies showing quadrivalent formation and heterozygote translocation.

The *Stipa* species studied possessed 2n = 24, 36 and 44 chromosome numbers. Taking x = 9, 11 and 12 as the basic chromosome numbers of the

Table 3. Ploidy level and cytogenetic abnormalities of the grass genera studied. CYTO – cytomixis, MP – multipolar formation, UNREDUCED – unreduced gamete formation, TRANS – heterozygote translocation, DIPLONT – diplontic behavior, B – B chromosome.

Species	x	CYTO	MP	UNREDUCED	TRANS	DIPLONT	B
Aegilops crassa	4x	+	–	–	–	+	–
A. crassa	6x	+	–	–	–	+	–
A. cylindrica	4x	+	–	–	–	+	+
A. tauschii	2x	+	–	–	–	–	+
A. speltoides	2x	+	–	–	+	–	–
A. triuncialis	4x	–	–	–	–	+	+
A. umbelulata	2x	+	–	–	–	+	–
Avena barbata	4x	+	+	–	–	+	+
A. eriantha	2x	+	–	–	–	+	+
A. eriantha	4x	–	–	–	–	+	+
A. fatua	6x	–	–	–	–	+	–
A. sterilis subsp. *ludviciana*	6x	+	–	–	–	+	–
A. wiestii	4x	+	–	–	–	+	–
Bromus brachystachys	2x	+	–	–	+	–	+
B. briziformis	2x	+	+	+	–	–	+
B. fasiculatus	4x	+	–	–	–	–	+
B. japonicus var. *japonicus*	2x	+	–	+	–	–	+
B. lanceolatus var. *lanceolatus*	4x		–	–	–	+	–
B. madreitensis	2x	+	–	–	–	–	–
B. rechingeri	4x	+	–	–	–	–	+
B. rubens	2x	+	–	–	–	–	–
B. scoparius var. *scoparius*	2x	+	–	–	–	–	–
B. sericeous	2x	–	–	–	–	–	–
B. sericeous	4x	–	–	–	–	–	–
B. squarrosus	2x	–	–	+	–	–	+
B. sterilis	6x	+	–	–	–	–	–
B. tectorum	4x	+	–	–	–	–	+
Festuca akhanii	6x	–	–	–	+	–	+
F. arundinaceae	6x	+	–	+	+	–	+
F. drymeja	2x	+	–	+	–	–	–
F. valesiaca	6x	+	–	+	+	–	–
Melica ciliata var. *ciliata*	2x	–	–	–	–	–	–
M. jacquemontii subsp. *canescens*	2x	–	–	–	–	–	–
M. jacquemontii subsp. *hohenackeri*	2x	–	–	–	–	–	–
M. jacquemontii subsp. *jacquemontii*	2x	–	–	–	–	–	–
M. persica subsp. *inaequiglumis*	2x	–	–	–	–	–	–
M. persica subsp. *persica*	2x	–	–	–		–	+
M. picta	2x	–	–	–	–	–	–
Milium schmidtiaum	2x	–	–	–	+	–	–
M. vernale	2x	–	–	–	–	–	–
M. vernale	4x	–	–	–	–	–	–
Secale cereale subsp. *ancestrale*	2x	–	–	–	–	–	–
S. cereale subsp. *cereale*	2x	+	–	–	+	–	+
S. strictum subsp. *strictum*	2x	+	–	–	+	+	–
Stipa arabica	4x	+	–	–	+	–	–
S. capensis	2x	+	–	–	+	–	–
S. caragana	4x	–	–	–	+	–	–
S. caucasica	4x	+	–	–	–	–	–
S. ehrenbergiana	4x	+	–	+	+	+	–
S. haussknechtii	4x	+	–	–	+	–	–
S. hohenackeriana	4x	+	–	–	–	–	–
S. holosericea	4x	+	–	–	–	–	–
S. iranica	4x	+	–	+	+	+	–
S. lessingiana	4x	+	–	–	+	–	–
S. parviflora	4x	+	–	–	+	–	–
S. pennata	4x	+	–	–	+	–	–
S. turkestanica	4x	+	–	–	–	–	–

genus *Stipa* (Freitag 1985), the species studied are diploid and tetraploid (4x). Freitag (1985) reported 2n = 40 for *S. turkestanica*, while the present study reports n = 22 (2n = 4x = 44) for this species. Vázquez and Devesa (1996) gave 2n = 2x = 28 for *S. parviflora*, while the present study reports n = 22 (2n = 4x = 44) for it. Two different chromosome numbers have been reported for other *Stipa* species, such as *S. leucotricha* Trin. & Rupr. (2n = 26 and 28; Löve & Löve 1948), *S. lemmonii* (Vasey) Scribn. (2n = 34 and 36; Stebbins & Löve 1941) and *S. bromoides* (L.) Dörfl. (2n = 24 and 28; Strid & Anderson 1985; Vázquez & Devesa 1996). This may suggest that along with hybridization and allopolyploidy, aneuploidy also played a role in the speciation of the genus *Stipa*. However, the mechanism(s) producing aneuploidy in the genus *Stipa* is not known.

Tetraploid species of *S. arabica*, *S. capensis*, *S. caucasica*, *S. hohenackeriana*, *S. lessingiana*, *S. pennata*, and *S. turkestanica* showed diplontic behavior and formed only bivalents in metaphase of meiosis I, possibly due to their allopolyploid nature or chiasmata formation mechanisms. The species *S. ehrenbergiana* had the highest values of relative total (1.86) and terminal (1.70) chiasmata; the value of intercalary chiasmata was highest (0.27) in *S. caucasica* (Table 2).

The ANOVA test performed for the studied grass genera showed significant differences for all relative meiotic characters except for mean number of rod bivalents and univalents per cell (Table 4), indicating their genomic distinctness. The genus *Stipa* had significantly fewer ring bivalents per cell than the other genera studied; the genera *Bromus* and *Milium* had significantly higher total chiasmata per chromosome than the other genera studied; and *Bromus* and *Aegilops* had significantly more intercalary chiasmata per chromosome than the others (Table 5).

Chiasma formation (frequency and distribution) is under genetic control (Quicke 1993), and the significant differences in the relative values of chiasmata between the different grass genera studied may indicate a significant change in the number of genes controlling chiasma frequency and distribution.

As shown by ANOVA, the different basic numbers (x) in the genera studied did not differ significantly in their relative cytogenetic values.

Table 4. ANOVA of cytogenetic characters of the studied grass genera (df – degree of freedom, F – Fisher ratio, MS – mean square, Sig. – significant, SS – sum of squares; other abbreviations as in Table 2).

Character		SS	df	MS	F	Sig.
RBN	Between groups	0.505	8	0.063	5.434	0.001
	Within groups	0.546	47	0.012		
	Total	1.050	55			
RDN	Between groups	0.189	8	0.024	1.371	0.234
	Within groups	0.809	47	0.017		
	Total	0.997	55			
IN	Between groups	0.018	8	0.002	1.976	0.070
	Within groups	0.054	47	0.001		
	Total	0.073	55			
IVN	Between groups	0.000	8	0.000	2.355	0.032
	Within groups	0.000	47	0.000		
	Total	0.000	55			
IXN	Between groups	1.234	8	0.154	6.602	0.001
	Within groups	1.098	47	0.02		
	Total	2.331	55			
TXN	Between groups	1.113	8	0.139	3.565	0.003
	Within groups	1.833	47	0.04		
	Total	2.946	55			
TOXN	Between groups	1.713	8	0.214	4.756	0.001
	Within groups	2.116	47	0.04		
	Total	3.829	55			

Table 5. Descriptive statistics of cytogenetic characters of the studied grass genera (number of species studied in square brackets [], SEM – standard error of mean; other abbreviations as in Table 2).

Genus		RBN	RDN	IN	IVN	IXN	TXN	TOXN
	Mean	0.74	0.21	0.02	0.00	0.28	1.46	1.75
	SEM	0.03	0.03	0.01	0.00	0.05	0.10	0.06
Aegilops [6]	Minimum	0.63	0.12	0.00	0.00	0.16	1.01	1.47
	Maximum	0.86	0.32	0.05	0.00	0.53	1.69	1.93
	Range	0.23	0.20	0.05	0.00	0.36	0.68	0.46
	Mean	0.79	0.19	0.02	0.00	0.38	1.51	1.89
	SEM	0.04	0.04	0.01	0.00	0.03	0.07	0.06
Avena [5]	Minimum	0.70	0.04	0.00	0.00	0.28	1.25	1.71
	Maximum	0.96	0.30	0.05	0.00	0.47	1.77	2.14
	Range	0.26	0.26	0.05	0.00	0.19	0.52	0.42
	Mean	0.80	0.16	0.03	0.00	0.42	1.64	2.06
	SEM	0.03	0.02	0.02	0.00	0.07	0.04	0.08
Bromus [13]	Minimum	0.60	0.04	0.00	0.00	0.11	1.26	1.51
	Maximum	0.94	0.40	0.26	0.00	1.12	1.91	2.90
	Range	0.34	0.36	0.26	0.00	1.01	0.65	1.39
	Mean	0.80	0.18	0.01	0.00	0.27	1.52	1.78
	SEM	0.03	0.03	0.00	0.00	0.10	0.13	0.05
Festuca [4]	Minimum	0.75	0.08	0.00	0.00	0.04	1.15	1.64
	Maximum	0.90	0.21	0.03	0.00	0.49	1.74	1.90
	Range	0.16	0.14	0.03	0.00	0.45	0.59	0.26
	Mean	0.85	0.14	0.00	0.00	0.03	1.73	1.76
	SEM	0.04	0.04	0.00	0.00	0.00	0.08	0.08
Melica [7]	Minimum	0.65	0.02	0.00	0.00	0.02	1.25	1.29
	Maximum	0.98	0.35	0.01	0.00	0.06	1.91	1.93
	Range	0.33	0.33	0.01	0.00	0.04	0.66	0.64
	Mean	0.93	0.06	0.00	0.00	0.04	2.05	2.09
	SEM	0.03	0.03	0.00	0.00	0.00	0.17	0.18
Milium [2]	Minimum	0.88	0.00	0.00	0.00	0.03	1.85	1.88
	Maximum	1.00	0.11	0.00	0.00	0.05	2.39	2.44
	Range	0.12	0.11	0.00	0.00	0.02	0.54	0.56
	Mean	0.84	0.26	0.03	0.00	0.21	1.65	1.93
	SEM	0.02	0.16	0.00	0.00	0.09	0.03	0.05
Secale [3]	Minimum	0.81	0.06	0.01	0.00	0.03	1.60	1.83
	Maximum	0.89	0.57	0.04	0.02	0.34	1.70	2.00
	Range	0.08	0.51	0.03	0.02	0.31	0.10	0.17
	Mean	0.60	0.27	0.01	0.00	0.14	1.52	1.67
	SEM	0.04	0.05	0.00	0.00	0.02	0.05	0.04
Stipa [13]	Minimum	0.35	0.08	0.00	0.00	0.03	1.24	1.41
	Maximum	0.81	0.61	0.03	0.00	0.27	1.75	1.86
	Range	0.46	0.53	0.03	0.00	0.24	0.52	0.45
	Mean	0.77	0.19	0.02	0.00[1]	0.25	1.60	1.86
	SEM	0.02	0.02	0.00	0.00[2]	0.03	0.03	0.04
Total [53]	Minimum	0.35	0.00	0.00	0.00	0.02	1.01	1.29
	Maximum	1.00	0.61	0.26	0.02	1.12	2.39	2.90
	Range	0.65	0.61	0.26	0.02	1.10	1.38	1.61

[1] – 0.0006; [2] – 0.0003

Moreover, no significant correlation between the basic chromosome number (x) and relative cytogenetic characteristics was found, indicating that the relative values of chiasma frequency and chromosome pairing do not increase with an increase in the basic chromosome number. Therefore it may be suggested that the significant differences observed between the genera studied are due mainly to their genomic differences and not to polyploidy or differences in basic chromosome number.

Different suggestions of the basic chromosome number of grasses have been offered. For example,

Avdulov (1931) measured chromosome numbers in hundreds of grasses and speculated that the ancestral chromosome number of grasses was x = 12, with lower basic chromosome numbers derived by aneuploid reduction. Flovik (1938) proposed an ancestral basic number of x = 5, whereas Sharma (1979) suggested that the ancestral basic number was x = 6. Stebbins (1985) concluded that ancestral basic chromosome numbers of x = 5, 6 or 7 were equally probable, with higher species chromosome numbers derived either by polyploidy, polyploidy followed by aneuploidy, or combinations (hybridization) of basic numbers. The ancestral basic chromosome number of the grasses is uncertain, but many historical polyploid and/or aneuploid events are required to adequately explain the current distribution of basic chromosome numbers among grass taxa.

UNREDUCED GAMETE FORMATION

Among the species of the eight genera of Pooideae studied, the occurrence of potential unreduced gametes (pollen grains) was observed in three genera: *Bromus*, *Festuca* and *Stipa*, the details of which are given here.

Large (possibly 2n) pollen grains along with smaller (normal) ones were in three species: *Bromus japonicus* var. *japonicus*, *B. briziformis*, and *B. squarrosus* (Table 3). The mean diameter of normal (reduced) pollen grains was 24.6 μm in *B. japonicus* var. *japonicus*, while the mean diameter of unreduced pollen grains was 33.5 μm. The corresponding values were 26.14 and 41.70 μm in *B. briziformis*, and 25.64 and 35.40 μm in *B. squarrosus*. The *t*-test revealed the difference in size between the larger and reduced pollen grains to be significant ($p < 0.05$) in all three species. The frequency of potential unreduced pollen grains varied from 1.58% to 4.12% in the *Bromus* species studied.

A numerically unreduced diploid or 2n gamete is a meiotic product which bears the sporophytic rather than the gametophytic chromosome number. Such gametes result from abnormalities during either microsporogenesis (2n pollen) or megasporogenesis (2n eggs). Unreduced gametes are known to produce individuals with higher ploidy level through a process known as sexual polyploidization (Villeux 1985). Sexual polyploidization has been considered a major route to the formation of naturally occurring polyploids. Different cytological mechanisms are responsible for the production of 2n gametes, including premiotic doubling of the chromosomes, omission of the first and second meiotic division, post-meiotic division, abnormal spindle geometry, abnormal cytokinesis and desynapsis (Villeux 1985). Detailed cytological study of *Bromus* species showed that cytomixis (discussed before) and chromosome stickiness leading to anaphase I and II failure as well as multipolar cell formation are possible mechanisms of unreduced pollen grain formation.

Large (possibly 2n) pollen grains occurred at a frequency of 0.3–19% in *Stipa caragana*, *S. haussknechtii*, *S. holosericea*, *S. iranica* and *S. parviflora*, along with smaller (normal) pollen grains. The diameter of 2n pollen grains ranged from 41 to 60 μm, while that of normal pollen grains was 28–39 μm. The size difference between unreduced and reduced pollen grains was significant by the *t*-test ($p < 0.05$).

Detailed cytological study of *S. iranica* showed cytomixis to be a possible mechanism for the formation of aneuploid and 2n meiocytes; the latter might produce unreduced pollen in this species. In the other four *Stipa* species, cytomixis, anaphase I and II failure are responsible for 2n pollen grain formation.

Large (possibly 2n) pollen grains pollen grains) occurred along with smaller (normal) pollen grains in *Festuca arundinacea*, *F. drymeja* and *F. valesiaca*, which also showed the occurrence of cytomixis. Large pollen grains were present at *ca* 2% frequency in these species.

The mean diameter of normal (reduced) pollen grains was 15.5 μm in *F. arundinacea*, and that of unreduced pollen grains was 22.0 μm. The corresponding values were 14.0 and 23.8 μm in *F. valesiaca*, and 8 and 19 μm in *F. drymeja.* The *t*-test showed the size difference between larger and smaller pollen grains to be significant ($p < 0.001$). Detailed cytological study of *Festuca* species

pointed to cytomixis as the possible mechanism of unreduced pollen grain formation.

Chromatin and chromosome migration (cytomixis) occurred in different directions from early prophase to telophase II in most of the species of *Bromus*, *Stipa*, *Aegilops*, *Avena* and *Festuca*. In some cases, one or a few chromosomes migrated into a neighboring meiocyte, leading to the formation of aneuploid cells. In fact, several metaphase/diakinesis cells in these species had extra or missing chromosomes, showing aneuploidy. In some other cells, the cell wall between two adjacent meiocytes dissolved in some way (syncyte formation) and the whole chromosomes of one meiocyte migrated to the neighboring cell, forming a meiocyte with double the normal chromosome number. Syncyte formation was highest in *B. scoparius* var. *scoparius* (15.57%) and *F. drymeja* (12.60%). Such unreduced meiocytes may result in the formation of unreduced pollen grains.

Migration of chromatin material or chromosomes among adjacent meiocytes occurs through cytoplasmic connections and cytomictic channels, as well as through cell wall dissolution (Falistocco *et al.* 1995). Cytomixis is considered to be of less evolutionary importance, but it may lead to the production of aneuploid plants with certain morphological characteristics (Sheidai *et al*. 1993) or may produce unreduced gametes as reported in several grass species including *Dactylis* (Falistocco *et al.* 1995) and *Aegilops* (Sheidai *et al*. 1999). Unreduced gamete formation is of evolutionary importance, leading to the production of plants with higher ploidy level.

B CHROMOSOMES

B chromosomes were observed in some of the species of *Aegilops*, *Avena*, *Bromus*, *Festuca*, *Melica* and *Secale* (Table 3). The B chromosomes observed were much smaller than the A chromosomes, round in shape, and did not pair with the A chromosomes or among themselves. In several cases the Bs could move to the cell poles, and in some they lagged and were eliminated from the cell cycle.

B chromosomes show numerical polymorphism, and when present in high numbers they negatively affect the growth and vigor of plants; in low numbers they may benefit the plant possessing them (Camacho *et al*. 2000). In most species possessing B chromosomes we did not obtain enough cells to analyze the effects of the presence of B chromosomes on cytogenetic characteristics; in some species, however, we could perform the *t*-test, as presented below.

In *Bromus japonicus* var. *japonicus*, the presence of B chromosomes significantly increased the mean values of terminal, intercalary and total chiasmata, and significantly reduced the mean number of rod bivalents. Therefore it seems that B chromosomes increase genetic recombination in this species and that better chromosome pairing occurs in their presence, as the number of ring bivalents significantly increased. This in turn may lead to better chromosome segregation and higher pollen fertility. In two species, *Aegilops tauschii* and *Festuca akhanii*, no significant difference in chiasma frequency and distribution or in chromosome pairing was found between cells with and without B chromosomes.

B chromosomes are known to play a role in plant adaptation to various environmental conditions (Camacho *et al*. 2000); the significant increase of genetic recombination observed in species possessing B chromosomes may enhance the adaptability of these plants.

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APPENDIX

MATERIAL STUDIED AND CHROMOSOME NUMBERS FOUND

Aegilops crassa Boiss. – IRAN: Chaharmahal & Bakhteyari, Chaharmahal, 1998, *Abdol Hossein Totiayi* (HSBU 98298), n = 14; Ilam, 1998, *Abdol Hossein Totiayi* (HSBU 98302), n = 21.

Aegilops cylindrica Host – IRAN: Kerman, Baft, 1998, *Abdol Hossein Totiayi* (HSBU 98287), n = 14.

Aegilops speltoides Tausch. – IRAN: Khoozestan, Ahvaz, 2008, *Abdol Hossein Totiayi* (HSBU 98289), n = 7.

Aegilops tauschii Coss. – IRAN: Azadshahr, 2008, *Abdol Hossein Totiayi* (HSBU 98290), n = 7.

Aegilops triuncialis L. – IRAN: Kerman, Jiroft, 2008, *Abdol Hossein Totiayi* (HSBU 98276), n = 14.

Aegilops umbellulata Zhuk. – IRAN: Ilam, Eivanegharb, 2008, *Abdol Hossein Totiayi* (HSBU 98283), n = 7.

Avena barbata Pott *ex* Link. – IRAN: Fars, Shiraz, 2008, *Abdol Hossein Totiayi* (HSBU 411093), n = 14.

Avena eriantha Durieu. – IRAN: Golestan, Gonbad, 2008, *Abdol Hossein Totiayi* (HSBU 411094), n = 7. Golestan, Gonbad, 2008, *Abdol Hossein Totiayi* (HSBU 411095), n = 14.

Avena fatua L. – IRAN: Markazi, Mahallat, 2008, *Abdol Hossein Totiayi* (HSBU 411095), n = 21.

Avena sterilis subsp. *ludoviciana* (Durieu) M. Gillet & Magne – IRAN: Fars, Shiraz, 2008, *Abdol Hossein Totiayi* (HSBU 98414), n = 21.

Avena wiestii Steud. – IRAN: Markazi, Mahallat, 2008, *Abdol Hossein Totiayi* (HSBU 98409), n = 21.

Bromus brachystachys Hornung – IRAN: Mazandaran, Noor, 2004, *Nouroozi & Amini* (HSBU 98409), n = 7.

Bromus briziformis Fisch. & C. A. Mey. – IRAN: Khorasan, Tandooreh national park, 2004, *Assadi & Massoumi* (TARI 50831), n = 7.

Bromus fasciculatus Presl. – IRAN: Fars, Booshehr, 7 m, 2004, *Fadaei & Amiri* (HSBU 123), n = 14.

Bromus japonicus Thumb. var. *japonicus* – IRAN: Karaj, Rajaeishahr, 2004, *Nouroozi & Amini* (HSBU 2028), n = 7.

Bromus lanceolatus Roth var. *lanceolatus* – IRAN: Arak, Sanjan, Farajollah mountain, 2004, *Noori* (HSBU 2024), n = 14.

Bromus madritensis L. – IRAN: Fars, Marvdash road, Palayeshgah national park, 1700 m, 2004, *Fadaei & Hatami* (HSBU 470), n = 14.

Bromus rechingeri Melderis – IRAN: Tehran, Imamzadeh-Davood, 2004, *Nouroozi & Parivand* (HSBU 2023), n = 14.

Bromus rubens L. – IRAN: Hormozgan, 150 km from Bandar-Abbas towards Sirjan, Gahkom, 720 m, 2004, *Fadaei & Assadpoor* (HSBU 113), n = 14.

Bromus scoparius L. var. *scoparius* – IRAN: Azarbayejan, Heyran, 1450 m, 2004, *Nouroozi & Parivand* (HSBU 2027), n = 7.

Bromus sericeus Drobow – IRAN: Sistan & Balochestan, Zahedan, Nokabad, 1790 m, 2004, *Fadaei*

& Nasiri (HSBU 131), n = 7, Khorasan, Neysaboor, 2000, 1100 m, *Iranshahr* (TARI 19870), n = 14.

Bromus squarrosus L. – IRAN: Ardebil, Meshkinshahr, *Nouroozi & Parivand* (HSBU 2022), n = 7.

Bromus sterilis L. – IRAN: Kerman, Bam to Jiroft, 2140 m, 2004, *Fadaei*, *Khodashenas*, *Ghonchehei*, *Keykha* (HSBU 455), n = 7.

Bromus tectorum L. – IRAN: Sistan & Baloochestan, Zahedan, Khash, 1550 m, 2004, *Fadaei & Nasiri* (HSBU 1048), n = 7

Festuca akhanii Tzvelev – IRAN: Golestan, National park, 2007, *Bagheri-Shabestarei* (HSBU 2040), n = 21.

Festuca arundinacea Schreb. – IRAN: Tehran, Karaj road, 2007, *Bagheri-Shabestarei* (HSBU 2041), n = 21.

Festuca drymeja Mert. & Koch. – IRAN: Gorgan, Looh jungle, 2007, *Bagheri-Shabestarei* (HSBU 2042), n = 7.

Festuca valesiaca Schleich. *ex* Gaudin – IRAN: Golestan, National park, 2007, *Bagheri-Shabestarei* (HSBU 2041), n = 21.

Melica ciliata L. var. *ciliata* – IRAN: Mazandaran, Dozdbon, *Moghaddam* (HSBU 5018), n = 9.

Melica jacquemontii Decne. subsp. *canescens*– IRAN: Mazandaran, Poleh-Zangooleh, 2006, *Moghaddam* (HSBU 5007), n = 9.

Melica jacquemontii (Boiss.) Bor. subsp. *hohenackeri* – IRAN: Tehran, Polour, 2006, *Moghaddam* (HSBU 5011), n = 9.

Melica jacquemontii Decne. subsp. *jacquemontii* – IRAN: Tehran, 2006, *Moghaddam* (HSBU 5006), n = 9.

Melica persica Kunth subsp. *inaequiglumis* – IRAN: Tehran, Boomehen, 2006, *Moghaddam* (HSBU 5004), n = 9.

Melica persica Kunth subsp. *persica* – IRAN: Tehran, Imamzadeh-Davood, 2006, *Moghaddam* (HSBU 5000), n = 9.

Melica picta K. Koch. – IRAN: Azarbayejan, Arasbaran, Makidi, 2006, *Moghaddam* (HSBU 5000), n = 9.

Milium vernale M. Bieb. – IRAN: Mazandaran, Javaherdeh, 2006, *Moghaddam* (HSBU 5028), n = 7. Gilan, Asalem to Khalkhal, 2006, *Moghaddam* (HSBU 5025), n = 9.

Milium schmidtianum K. Koch. – IRAN: Azarbayejan, Arasbaran, 2006, *Moghaddam* (HSBU 5024), n = 7.

Secale cereale L. subsp. *ancestrale* Zhuk. – IRAN: Ghazvin, Eghbalieh, 2007, *Ali-Jarrahi*, (HSBU 5024), n = 7.

Secale cereale L. subsp. *cereale* – IRAN: Ghazvin, Zibashahr, 2007, *Ali-Jarrahi*, (HSBU 3750), n = 7.

Secale strictum C. Presl subsp. *strictum* – IRAN: Tehran, Damavand, 2007, *Ali-Jarrahi*, (HSBU 3575), n = 7.

Stipa arabica Trin. & Rupr. – IRAN: Tehran, 2005, *Attaei*, (HSBU 3600), n = 22.

Stipa capensis Thunb. – IRAN: Gilan, Loshan, 2005, *Khoseavi-Reineh*, (HSBU 3601), n = 18.

Stipa caragana Trin. – IRAN: Tehran, Firoozkooh road, 2005, *Attaei*, (HSBU 3602), n = 12.

Stipa caucasica Schmalh – IRAN: Golestan, Gorgan, 2005, *Attaei*, (HSBU 3603), n = 22.

Stipa ehrenbergiana Trin. & Rupr. – IRAN: Azarbayejan, Meyaneh, 2005, *Khoseavi-Reineh*, (HSBU 3604), n = 22.

Stipa haussknechtii Boiss. – IRAN: Isfahan, Shahreza, Kolah-Ghazi, 1700 m, 2005, *Khoseavi-Reineh*, (HSBU 3605), n = 22.

Stipa hohenackeriana Trin. & Rupr. – IRAN: Azarbayejan, Meshkinshahr, 2005, *Khoseavi-Reineh*, (HSBU 3606), n = 22.

Stipa holosericea Trin. – IRAN: Tehran, Damavand, 2005, *Khoseavi-Reineh*, (HSBU 3607), n = 22.

Stipa iranica Freitag – IRAN: Tehran, 2005, *Khoseavi-Reineh*, (HSBU 3608), n = 22.

Stipa lessingiana Trin. & Rupr. – IRAN: Tehran, 2005, *Khoseavi-Reineh*, (HSBU 3608), n = 22.

Stipa parviflora Desf. – IRAN: Markazi, Mahallat, 2005, *Khoseavi-Reineh*, (HSBU 3609), n = 22.

Stipa pennata L. – IRAN: Golestan, Dashte-Gorgan, 2005, *Khoseavi-Reineh*, (HSBU 3610), n = 22.

Stipa turkestanica Hackel – IRAN: Tehran, 2005, *Khoseavi-Reineh*, (HSBU 3611), n = 22.