Meiotic Chromosome Numbers in 9 Iranian Species of *Oryzopsis* (Poaceae)

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Summary Meiotic studies were performed in 9 *Oryzopsis* species of *O. pubiflora*, *O. lateralis*, *O. holciformis*, *O. barbellata*, *O. munroi*, *O. microcarpa*, *O. sphacelata*, *O. virescense* and *O. molinioides* showing the presence of $2n=2x=24$ in all nine species studied. The species studied were diploid and had 12 chromosome number, and in *O. microcarpa*, *O. sphacelata*, *O. molinioides* and *O. virescense* chromosomes are new count. B-chromosomes 0–2 were observed in all species studied, which were smaller than A-chromosomes and did not pair with them. T-test analysis between the cells with B-chromosomes and those devoid of Bs showed significant difference in chiasma frequency and distribution as well as in chromosomes pairing only in *O. munroi*, with significant reduction in meiotic values in the presence of B-chromosomes. The occurrence of large pollen grains (possibly unreduced pollen grains) was observed along with small (normal) pollen grains in these species. Significant difference observed in chiasma frequency and distribution among species studied may be of use in species delimitation. Moreover species relationships obtained based on cytological data partly supports taxonomic treatment of the genus.

Key words B-chromosome, Chiasma frequency, Meiotic chromosome number, *Oryzopsis*.

Rice grass (*Oryzopsis* Michx.) is a genus with about 35 open habitats species which are hermaphroditic, caespitose, tufted perennials, growing in temperate and subtropical regions of northern hemisphere and Middle East, occupying mainly dry mountain slopes. However, a few species occur in woodland (Watson and Dallwitz 1992). *Oryzopsis* species show close affinity and frequently hybridize with the *Stipa* species (Johnson 1945a, 1945b, 1960, 1962, 1963, Hitchcock and Spellenberg 1968, Shechter 1969, Spellenberg and Mehlenbacher 1971). About 10 species are reported from Iran (Bor 1970). The chromosome base numbers of the genus *Oryzopsis* are $x=11$, 12, and 14. Diploid, tetraploid, pentaploid and even higher ploidy levels have been reported in this genus (Watson and Dallwitz 1992).

Cytotaxonomical studies have been considered useful in showing species relationships, taxonomic delimitation and genetic differences in several plant groups including grass genera of *Aegilops* (Sheidai *et al.* 1999, 2000, 2002), *Bromus* (Stebbins 1981, Sheidai and Nouroozi 2005, Sheidai and Fadaei 2005, Sheidai *et al.* 2008), *Stipa* (Stebbins 1998, Sheidai and Attai 2005, Sheidai *et al.* 2006), *Festuca* (Sheidai and Bagheri-Shabestarei 2007a), and *Hordeum* (Sheidai and Rashid 2007), as well as in other plant groups like *Asparagus* (Sheidai and Inamdard 1997), *Hyoscyamus* (Sheidai *et al.* 1999) and *Echinops* (Sheidai *et al.* 2000).

Cytological reports on *Oryzopsis* species are very limited (Johnson and Rogler 1943, Johnson 1945a, 1945b, 1960, 1962, 1963, Hitchcock and Spellenberg 1968, Shechter 1969, Spellenberg and Mehlenbacher 1971, Sharma and Sharma 1979, Love and Love 1981a) and there is only 1 report from Iran dealing with chromosome pairing in 4 species only (Yazdanbakhsh *et al.* 2010).

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Table 1. Meiotic characteristics in *Oryzopsis* species

Species	Locality	TX	IX	TOX	RII	RD	I
<i>O. monilioides</i>	Fars, Bel montain	20.70	2.50	23.20	9.85	1.20	1.90
<i>O. munroi</i>	Khorasane razavi, Kalate	21.47	4.50	26.00	10.80	0.02	2.20
<i>O. sphacelata</i>	Fars, Bazbache	17.00	6.00	23.10	8.30	0.37	6.58
<i>O. lateralis</i>	Fars, Bel mountain	18.70	4.00	22.80	8.90	1.32	3.57
<i>O. holciformis</i>	Mazandaran, Rozan	23.40	2.40	25.80	11.60	0.20	0.40
<i>O. virescense</i>	Mazandaran, Taker	19.50	1.90	21.40	9.50	0.50	3.70
<i>O. microcarpa</i>	Khorasane razavi, Ghonchi	23.50	2.00	25.40	11.70	0.10	0.40
<i>O. pubiflora</i>	Hanna lake Esfahan	13.78	4.44	18.23	6.94	0.24	9.42
<i>O. barbellata</i>	Tehran, Touchal mountain	22.30	7.30	29.70	11.30	0.17	0.33

Abbreviations: TX=Mean number of terminal chiasmata, IX=Mean number of intercalary chiasmata, TOX=Mean number of total chiasmata, RB=Mean number of ring bivalents, RD=Mean number of rod bivalents, I=Mean number of univalents.

Table 2. ANOVA test of meiotic characteristics in *Oryzopsis* species

		Sum of Squares	df	Mean Square	F	Sig.
TX	Between Groups	144.343	6	24.057	4.077	.005
	Within Groups	165.200	28	5.900		
	Total	309.543	34			
IX	Between Groups	72.571	6	12.095	4.980	.001
	Within Groups	68.000	28	2.429		
	Total	140.571	34			
TOX	Between Groups	144.286	6	24.048	2.256	.067
	Within Groups	298.400	28	10.657		
	Total	442.686	34			
RB	Between Groups	45.600	6	7.600	4.092	.005
	Within Groups	52.000	28	1.857		
	Total	97.600	34			
RD	Between Groups	7.886	6	1.314	1.769	.142
	Within Groups	20.800	28	.743		
	Total	28.686	34			
I	Between Groups	135.771	6	22.629	3.845	.006
	Within Groups	164.800	28	5.886		
	Total	300.571	34			

TX=Mean number of terminal chiasmata, IX=Mean number of intercalary chiasmata, TOX=Mean number of total chiasmata, RB=Mean number of ring bivalents, RD=Mean number of rod bivalents, I=Mean number of univalents.

Therefore the present study considers ploidy level, chromosome pairing and meiotic characteristics of 9 *Oryzopsis* species growing wild in Iran for the first time.

Materials and methods

Plant material

In our plant material collections, young flower buds could be obtained for 9 *Oryzopsis* species growing in Iran (Table 1), namely: *O. pubiflora* Hack., *O. lateralis* (Regel) Stapf., *O. holciformis* (M.B) Hack., *O. barbellata* (Mez) Bor., *O. munroi* Stapf, *O. microcarpa* Pilger, *O. sphacelata* (Boiss. and Buhse) Hack., *O. virescense* (Trin.) Beck. and *O. molinioides* (Boiss.) Hack. Ex Paulsen. The voucher specimens are deposited in the Herbarium of Shahid Beheshti University (HSBU).

Fig. 1. Representative meiotic cells in *Oryzopsis* species. A: Metaphase I cell showing bivalents in *O. microcarpa*. B: Diakinesis cell showing bivalents in *O. munroi*. C: Metaphase I cell showing B-chromosome (arrow) in *O. munroi*. D–F: Metaphase I cells showing bivalents in *O. holciformis*, *O. virescens* and *O. sphacelata* respectively. G–I: Meiotic cells showing stickiness, laggard chromosomes, anaphase bridge and telophase II laggards in *O. munroi* respectively. K and L: Potential unreduced pollen grains (larger pollen grains) in *O. munroi* and *O. monilioides* respectively. Scale bar=10 μ m.

Cytological studies

Meiotic studies were performed on young flower buds collected from at least 10 randomly selected plants from each species and population. A minimum of 100 metaphase/diakinesis pollen mother cells (PMCs) and 500 anaphase and telophase cells were analysed for data collection (Sheidai and Rashid 2007). Pollen stainability as a measure of fertility was determined by staining minimum 1000 pollen grains with 2% acetocarmine: 50% glycerin (1:1) for about 1/2 h. Round complete pollens which were stained were taken as fertile, while incomplete, shrunken pollens with no stain were considered as infertile (Sheidai and Rashid 2007).

Statistical analyses

An analysis of variance (ANOVA), followed by a Least Significant Test (LSD), was performed to detect any significant difference in chiasma frequency and chromosome pairing among the species studied (Sheidai and Rashid 2007). A t-test analysis was performed to study size difference between normal and large pollen grains. At least 50 large pollen grains and 50 small pollen grains were randomly measured for t-test analysis. This t-test analysis was performed to detect any significant difference in chiasma frequency and chromosome pairing among the cells with and without B-chromosomes. Grouping of the species was done by using different clustering methods, including Unweighted Paired Group with Arithmetic Average (UPGMA) and Neighbor Joining

Fig. 2. UPGMA dendrogram of *Oryzopsis* species based on cytological data.

Fig. 3. PCA plot of *Oryzopsis* species based on cytological data.

(NJ) as well as ordination plot based on Principal Components Analysis (PCA) (Sheidai and Rashid 2007). Statistical analyses used SPSS ver. 9 (1998), NTSYS ver. 2.2 (1998) and DARwin ver. 5. (2000) software.

Results

Chromosome number and chiasma frequency

The species and populations studied were diploid and possessed $2n=2x=24$ chromosome number (Table 1, Fig. 2A–I), supporting the earlier reports on *O. holciformis* var. *holciformis*, *O. holciformis* var. *longiglumis*, *O. molinioides*, *O. barbellata*, *O. pubiflora*, *O. munroi* and *O. lateralis*

(Sharma and Sharma 1979, Nasirzadeh *et al.* 2004, Sheidai *et al.* 2010). To our knowledge the chromosome number of *O. microcarpa*, *O. sphacelata*, *O. molinioides* and *O. virescense* are new to science.

O. barbellata showed the highest value of intercalary and total chiasmata as well as ring bivalents (7.30, 29.70 and 11.30 respectively), while *O. microcarpa* had the highest value of terminal chiasmata (23.50). The lowest value of total chiasmata and ring bivalents occurred in *O. pubiflora* (18.23 and 6.94 respectively), while the lowest value of intercalary chiasmata occurred in *O. virescense* (1.90). Univalents were formed in all the species and populations studied with *O. pubiflora* showing the highest mean value (9.42) and *O. barbellata* showing the lowest value (0.33).

Oryzopsis species studied showed a high percentage of pollen fertility (about 100%). The ANOVA test (Table 2) showed a significant difference ($p < 0.05$) in chiasma frequency and distribution as well as chromosome association among *Oryzopsis* species studied.

Laggard chromosomes were observed in anaphase of meiosis-I in *O. munroi*, *O. molinioides* and *O. lateralis* (Fig. 1H), ranging from 1.10–3.82%. Such laggards may have formed micronuclei observed in these species (Fig. 1J). Metaphase and anaphase II stickiness occurred in *O. microcarpa* and *O. holciformis* (Fig. 1I).

Grouping of the *Oryzopsis* species, based on cytological data obtained by different clustering and ordination methods, produced similar results (Figs. 2, 3), showing similarities between *O. molinioides*, *O. lateralis* and *O. virescense* which is not supported by taxonomic treatment of the genus in Flora Iranica (Bor 1970), as these 3 species have been placed far from each other.

The second cluster or group identified shows close affinity among *O. holciformis*, *O. microcarpa*, *O. munroi* and *O. barbellata*, with higher similarity between the first 2 species. Such species affinities have been also suggested by Bor (1970) in his taxonomic treatment of the genus. Both cluster and ordination results suggest close relationship between *O. sphacelata* and *O. pubiflora*, which is also suggested in Flora Iranica.

B-chromosomes

B-chromosomes (Bs) of 0–2 were observed in all species studied (Fig. 1C), which were smaller than A-chromosomes and did not pair with them. Bs could arrange them in the equator and move to the poles being included in the telophase nucleus. This is the first report on the occurrence of Bs in *O. microcarpa*, *O. sphacelata*, *O. molinioides* and *O. virescense*.

The t-test analysis between the cells with Bs and those devoid of Bs showed significant differences in chiasma frequency and distribution as well as in chromosomes pairing only in *O. munroi*, with significant reduction in meiotic values in the presence of Bs.

Large pollen grains

The occurrence of large pollen grains (possibly $2n$ pollen grains) was observed along with smaller (normal) pollen grains in *O. munroi*, *O. monilioides* and *O. virescense* (Fig. 1K and L). 2% of the analysed pollen grains are large in these species. The size of small (reduced) pollen grains ranged from 27 to 31 μm (mean=29 μm) in *O. munroi*, while the size of larger pollen grains (reduced) ranged from 35 to 45 μm (mean=40 μm). Similarly, the size of small (reduced) pollen grains ranged from 30 to 36 μm (mean=33 μm) in *O. monilioides*, while the size of larger pollen grains (reduced) ranged from 38 to 41 μm (mean=39.5 μm). In *O. virescense*, small pollen grains ranged from 32–38 μm (mean=36 μm) and larger pollen grains ranged in size from 39 to 46 μm (mean=43 μm). A t-test analysis of the size of pollen grains showed that larger pollen grains differ significantly from the small pollen grains ($p < 0.05$).

Discussion

Variations reported in the chromosome numbers of *Oryzopsis* species indicate the presence of 2 basic chromosome numbers of $x=11$ and $x=12$ in the genus and the possible role played by aneuploidy and chromosome loss/gain during the species diversification.

Significant differences observed in chiasma frequency and localization among *Oryzopsis* species studied may indicate partly their genetic difference (Quicke 1993) and possible significant change in the genes controlling chromosome pairing in the genus. Such variations have been reported in populations of different species (Rees and Jones 1977), and are considered as a means for generating new forms of recombination influencing the variability within natural populations in an adaptive way (Rees and Jones 1977). As stated before, different species of *Oryzopsis* can cross spontaneously, which could give rise to intermediate types adding to the species diversity and variability in the genus. The results of clustering and ordination plots showed that cytological data might be of use in *Oryzopsis* species delimitation and revealing the species relationships.

Bs are reported in more than 1300 species of plants and almost 500 species of animals (Camacho *et al.* 2000). The occurrence of Bs in all the species and populations studied may indicate the role played by these chromosomes in species diversification and adaptation. However, the present analyses only showed a significant effects on chiasma frequency and distribution in 1 species, but even slight non-significant changes may have some effects on the plants possessing them. Our previous studies on the effect of Bs on chiasma frequency and distribution as well as chromosome pairing in grass species have indicated a significant increase in *Bromus* and *Aegilops* species (Sheidai *et al.* 1999, Sheidai and Nouroozi 2005).

The occurrence of significantly larger pollen grains in some of the *Oryzopsis* species may indicate the occurrence of potential unreduced pollen grains in these species, which has also been reported before for *O. lateralis*, *O. pubiflora* and *O. barbellata* (Yazdanbakhsh *et al.* 2010).

Unreduced gametes are known to produce individuals with a higher ploidy level through a process known as sexual polyploidization (Villeux 1985), which has been considered the major route to the formation of naturally occurring polyploids. Such polyploid plants may grow in the vicinity of diploid parental plants, but in our study the species and populations studied showed diploid chromosome number and polyploid plants/species were not identified by us. This may indicate that the low percentage of unreduced pollen grains formed in the *Oryzopsis* species have not been successful in producing polyploid plants till now, although we may encounter polyploid populations in our future explorations.

Different cytological mechanisms are responsible for the production of $2n$ gametes (Bertagnolle and Thomson 1995). Anaphase failure, cytomixis and multipolar cell formation have been reported as the main cytological mechanisms of unreduced gamete formation in some grass species including *Bromus*, *Festuca* and *Stipa* (Sheidai and Nouroozi 2005, Sheidai and Bagheri-Shabestari 2007b, Sheidai *et al.* 2006). The occurrence of large number of univalents and the failure of chromosome movement to the poles as well as multipolar cell formation may be considered as the possible mechanisms of unreduced meiocyte formation in the *Oryzopsis* species as suggested before (Yazdanbakhsh *et al.* 2010). Therefore it seems that different cytological mechanisms, including changes in the chromosome number, significant changes in the number of genes controlling chromosome pairing, the occurrence of Bs and unreduced pollen grain formation along with hybridization, may have played a role in the evolution and diversification of the *Oryzopsis* species.

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