RESEARCH NOTE

Cytogenetic studies in some species of *Bromus* L., section *Genea* Dum.

MASOUD SHEIDAI* and FATEMEH FADAEI

Biology Department, Shahid Beheshti University, Tehran, Iran

Introduction

The genus *Bromus* L. (tribe Bromeae, family Poaceae) comprises about 160 annual and perennial species (Acedo and Liamas 2001), distributed all over the world. *Bromus* species are among important range grasses of Iran and are placed in six sections, of which section *Genea* Dum. contains six perennial species found in Iran (Bor 1970). The available literature dealing with cytogenetics of *Bromus* (e.g. Devesa *et al*. 1990; Lövkvist and Hultgård 1999), indicates the importance of such cytological studies for understanding the evolution of the genus *Bromus*. Therefore, we studied chiasma frequency and distribution, as well as chromosomal association and segregation, in ten Iranian populations of six *Bromus* species from the section *Genea*. The results uncovered several hitherto undescribed inter-population variations in cytological characteristics.

Materials and methods

Plant material

We studied ten populations of six *Bromus* species: *B. tectorum* L. (two populations), *B. sericeus* Drobov. (two populations), *B. madritensis* L. (one population), *B. rubens* L. (two populations), *B. fasciculatus* Presl. (one population), and *B. sterilis* (two populations). Voucher specimens are deposited in the Herbarium of Shahid Beheshti University (HSBU) and Herbarium of Iran Botanical Garden (TARI).

Cytological preparation and meiotic analysis

Young flower buds were collected from ten randomly selected plants of each population, fixed in glacial acetic acid: ethanol (1 : 3) for 24 h, and then washed and preserved in 70% ethanol at 4°C until used, following Sheidai *et al*. (2003). Cytological preparations used squash technique and 2% aceto-orcein as the stain.

Between 50 and 100 pollen mother cells (PMCs) were analysed for chiasma frequency and distribution at diakinesis/metaphase stage, and 500 PMCs were analysed for chromosome segregation during the anaphase and telophase stages. Pollen stainablity, as a measure of fertility, was determined by staining a minimum of 1000 pollen grains with 2% acetocarmine: 50% glycerin (1 : 1) for about 30 min. Round/complete pollens which were stained were taken as fertile, while incomplete/shrunken pollens with no stain were considered as infertile (Sheidai *et al*. 2003).

Results and discussion

Overall, the *Bromus* species studied here showed pollen fertility of 88% (*B. rubens*) to 99% (*B. tectorum*). The possible reasons for a low reduction of pollen fertility in *Bromus* species may be chromosome stickiness, laggard formation and cytomixis.

Chromosome number and chiasmata

Both populations of *B. tectorum* possessed $n = 7$ ($2n = 2x = 14$) chromosome number (figure 1 b,d), supporting an earlier report (Lövkvist and Hultgård 1999; but see also Devesa *et al*. 1990). Although more total and terminal chiasmata as well as ring bivalents were observed in Zahedan population of *B. tectorum* compared to the Fars population (table 1), the differences were not significant ($t = 1.18$, $P = 0.30$). The two populations of *B. sericeus* studied differed in their ploidy level. The Iranshahr population possessed $n = 14$ ($2n = 4x = 28$) chromosome number (figure 1c), while the Khash population possessed $n = 7$ ($2n = 2x = 14$). The earlier study on this species reports the somatic chromosome number of $2n = 14$ (Bolkovskikh *et al*. 1969). Therefore, this is the first report on among-population variation in ploidy level of *B. sericeus*.

*For correspondence. E-mail: msheidai@yahoo.com

Keywords. B-chromosomes; *Bromus*; chiasma frequency; cytomixis.

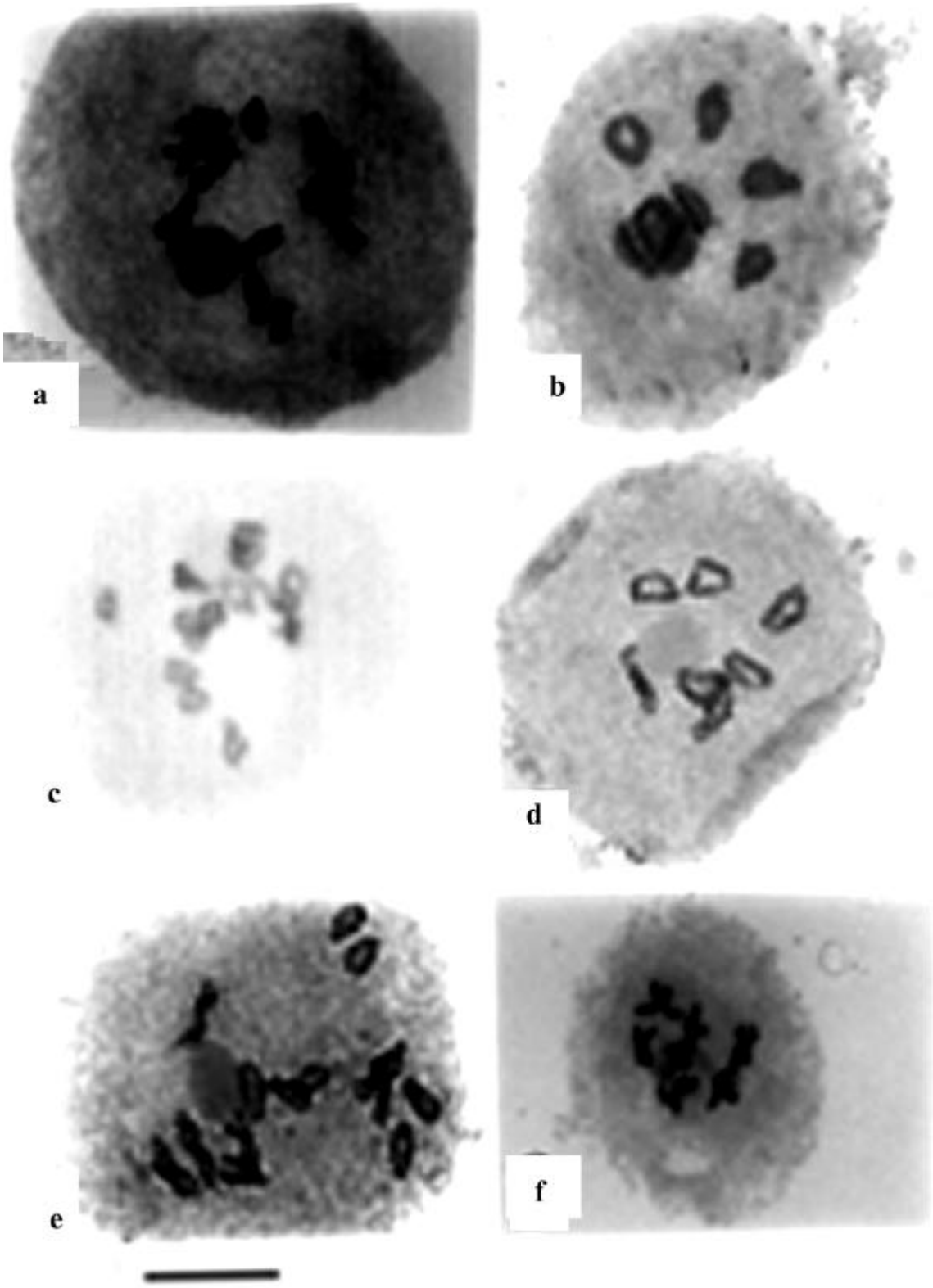

Figure 1. Representative meiotic cells in *Bromus* species. (**a**) *B. rubens* showing $n = 14$; (**b**) *B. tectorum* (Zahedan population) showing $n = 7$; (**c**) *B. sericeus* (Iranshahr population) showing $n = 14$; (**d**) *B. tectorum* (Fars population) showing $n = 7$; (**e**) *B. fasciculatus* (Booshehr population) showing $n = 14$; (**f**) *B. sterilis* (Kerman population) showing $n = 7$ (scale bar = 10 μm).

Iranshahr population of *B. sericeus* which is tetraploid, formed only bivalents in metaphase-I (table 1). This is considered to be a cytogenetic characteristic of true allopolyploids, however at present we are not sure if it is a true allopolyploid or the formation of only bivalents is due to presence of a diplodizing mechanism as present in other grasses (Sybenga 1992).

Two populations of *B. rubens* studied possessed $n = 14$ ($2n = 4x = 28$; figure 1a, table 1), supporting the earlier report of Vogt and Aparicio (1999). Although more total and terminal chiasmata as well as ring bivalents were observed in Hajiabad population (28.54, 23.83, 5.16 and 11.66 respectively; table 1) compared to that of Gahkom population, *t*-tests did not show significant differences between the two populations. Both populations formed a low number of (0.13–0.27) quadrivalents (table 1).

Two populations of *B. sterilis* studied possessed $n = 7$ ($2n = 2x = 14$; figure 1f, figure 2b, table 1), supporting the earlier report of Lövkvist and Hultgård (1999). Although a higher mean number of total and intercalary chiasmata as well as ring bivalents were observed in Fars population (13.72, 2.28 and 5.56 respectively; table 1) compared to that of Kerman population, *t*-tests did not show a significant differences between the two populations. A tetraploid ($2n = 4x = 28$; Dobeš *et al*. 1997) and octaploid ($2n = 8x = 56$; Devesa *et al*. 1990) chromosome number has been also reported for *B. sterilis*. The *B. sterilis* populations studied here are diploid and are expected to form bivalents and univalents. Yet, a very low number of quadrivalents (0.04) were observed in Kerman population (table 1), possibly due to the occurrence of heterozygote translocation.

The only population of *B. madritensis* studied showed the presence of $n = 14$ ($2n = 4x = 28$) chromosome number, supporting the report of Esnault (1984). This species is tetraploid but showed diplontic behavior, forming only bivalents due to its allopolyploid nature (Sales 1994).

The only population of *B. fasciculatus* studied showed the presence of $n = 14$ ($2n = 4x = 28$; figure 1e) chromosome number. The only report previously available for this species shows the presence of $2n = 2x = 14$ (Napoli and Zizza 1984). Therefore, the present study reports a new ploidy level ($4x$) for *B. fasciculatus*, Bivalents and quadrivalents were formed in metaphase and diakinesis stages of meiosis-I in this species.

Variation in chiasma frequency and localization is genetically controlled (Coucoli *et al*. 1975) and has been reported in populations of different grass species like *Aegilops*, *Lolium* and *Festuca* (Rees and Dale 1974). Such a variation in the species/populations with the same chromosome number is considered as a means for generating different kinds of recombinants, influencing the variability within natural populations in a possibly adaptive manner (Rees and Dale 1974).

We have earlier shown the presence of a specific control over chiasma frequency and distribution in *Stipa* (Poaceae) species having different chromosome numbers (Sheidai *et al*. 2003). In order to check if a similar control exists in the *Bromus* species having $2n = 14$ and 28, the plot of relative chiasmata values (chiasmata/chromosome number) was sketched against different chromosome numbers, and revealed no difference in relative chiasmata value between species having $n = 7$ and $n = 14$. The correlation test performed between chromosome number and relative chiasmata values in *Bromus* species also did not show a significant correlation.

Sticky chromosomes

Sticky chromosomes were observed from early stages of prophase and continued to the final stages of meiosis in

Table 1. Meiotic characters in *Bromus* species studied.

Sp	2*n*	TX	IX	TOX	RB	RD	Q	I	TXN	IXN	TOXN	RBN	RDN	QN	IN	PF	B
tect1	14	10.96	2.29	13.26	5.67	1.14	0.00	0.14	1.57	0.33	1.89	0.81	0.16	0.00	0.02	98.36	0–2
tect2	14	0.60	2.60	13.20	5.20	1.40	0.00	0.20	0.09	0.37	1.89	0.74	0.20	0.00	0.03	99.22	0–1
ser1	28	24.33	7.16	31.50	12.83	0.83	0.00	0.33	1.74	0.51	2.25	0.92	0.06	0.00	0.02	89.63	0
ser2	14	8.80	1.80	10.60	4.60	0.60	0.00	1.80	1.26	0.26	1.51	0.66	0.09	0.00	0.26	92.08	0
mad	28	24.11	5.88	30.00	12.55	1.22	0.00	0.22	1.72	0.42	2.14	0.90	0.09	0.00	0.02	93.02	0–2
rub1	28	23.27	2.63	25.90	10.56	2.54	0.27	0.27	1.66	0.19	1.85	0.75	0.18	0.02	0.02	96.74	0
rub2	28	23.83	5.16	28.54	11.66	2.08	0.13	0.00	1.70	0.37	2.04	0.83	0.15	0.01	0.00	88.15	0–2
fas	28	20.16	4.95	25.08	10.50	2.33	0.08	0.95	1.44	0.35	1.79	0.75	0.17	0.01	0.07	95.20	0
ster1	14	11.44	2.28	13.72	5.56	2.28	0.00	0.16	1.63	0.33	1.96	0.79	0.33	0.00	0.02	90.12	0–1
ster2	14	11.70	0.97	12.64	5.10	1.77	0.04	0.04	1.67	0.14	1.81	0.73	0.25	0.01	0.01	96.31	0

TX = terminal chiasmata; IX = intercalary chiasmata; TOX = total chiasmata; RB = ring bivalent; RD = rod bivalent; Q = quadrivalent; I = univalent; TXN = terminal chiasmata/bivalent; IXN = intercalary chiasmata/bivalent; TOXN = total chiasmata/bivalent; RBN = ring bivalent/cell; RDN = rod bivalent/cell; QN = quadrivalent/cell; IN = univalent/cell; PF = pollen fertility; B = number of B-chromosomes.

Species code: tect 1 & 2 = *B. tectorum* Zahedan and Fars populations, respectively; ser 1 & 2 = *B. sericeus* Iranshahr and Khash populations, respectively; mad = *B. madritensis* Fars population; rub 1 & 2 = *B. rubens* Gahkom and Hajiabad populations, respectively; fas = *B. fasciculatus* Booshehr population; ster 1 & 2 = *B. sterilis* Fars and Kerman populations, respectively.

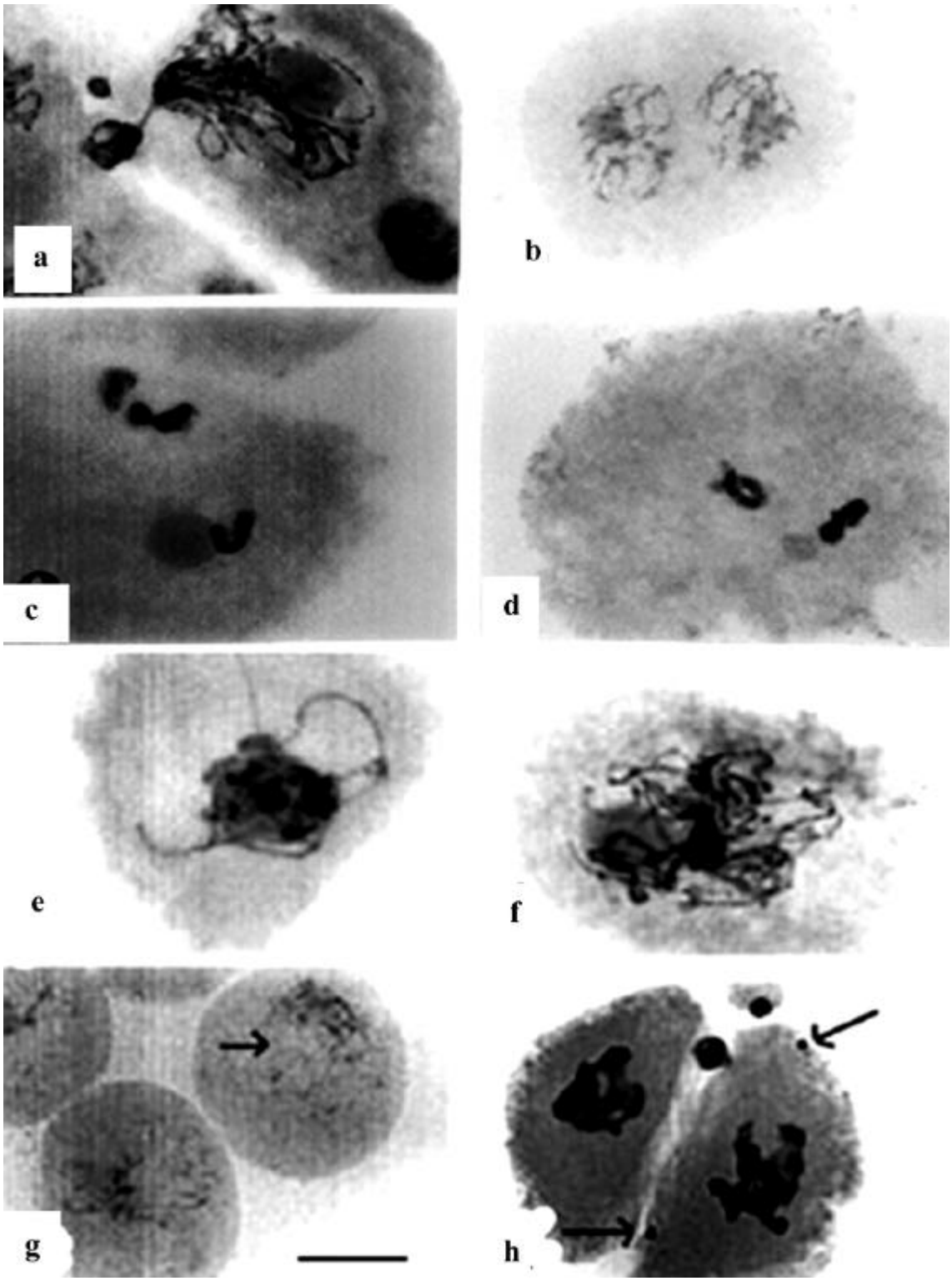

Figure 2. Representative meiotic cells in *Bromus* species. (**a**) Chromosome migration in *B. fasciculatus*; (**b**) cytomictic cells showing double chromosome number in *B. sterilis*; (**c**) a meiocyte showing reduction in chromosome number in *B. fasciculatus*; (**d**) a meiocyte showing reduction in chromosome number in *B. sterilis* (Fars population); (**e**) synezetic knot stage in *B. fasciculatus*; (**f**) pachytene in *B. fasciculatus*; (**g**) diffuse stage (arrows) in *B. tectorum*; (**h**) B-chromosomes (arrows) in *B. tectorum* (Fars population) (scale bar = 10 μm).

most of the species studied (table 1). The number of chromosomes involved in stickiness varied from two to many, often forming a complete clumping of the chromosomes. Stickiness varied among different meiocytes and the species studied. Such a phenomenon has also been reported in *Avena* species (Baptista *et al*. 2000; Sheidai *et al*. 2003). Genetic and environmental factors (Nirmala and Rao 1996), as well as genotype × environment interactions (Baptista *et al*. 2000), have been implicated as reasons for chromosome stickiness in different plant species, and this may be true for the *Bromus* species studied here as well.

B-chromosomes

B-chromosomes are accessory chromosomes reported in more than 1300 species of plants including, some *Bromus* species, and almost 500 species of animals (Camacho *et al*. 2000). The B-chromosomes show numerical polymorphism and, when present in high number, can negatively affect the growth and vigour of the plants, while in low number they may benefit the plant. B-chromosomes (0–2) were observed in some of the *Bromus* species and populations studied here (table 1, figure 2h). These chromosomes were smaller than the A-chromosomes and did not form any association with them. They could arrange themselves along with the A-chromosomes on the equatorial plane and move to the poles during anaphase. In some cases, they would lag in anaphase leading to their elimination from the cell. The occurrence of B-chromosome in *B. tectorum* and *B. sterilis* is reported here for the first time.

The occurrence of B-chromosomes in only one population of *B. sterilis* shows inter-population variation in the occurrence of B-chromosomes. It is possible that the lagging of B-chromosomes in anaphase cells of *Bromus* spp. may help the plants possessing them to exercise some control over the accumulation of B-chromosomes which may negatively affect them. Due to the low number of meiocytes showing presence of B-chromosomes in *Bromus* species studied, their effects on chiasma frequency and chromosome associations could not be worked out.

Diffuse stage in meiosis-I prophase

The meiotic analysis of all *Bromus* species studied showed a deviant course of meiosis-I prophase sub-stages i.e. the occurrence of synezetic knot stage instead of leptotene and zygotene (figure 2e). In the early synezetic knot stage, thin chromatin strands surround the nucleolus, eventually covering it totally. Later on, paired chromosomes unraveled from the knot, entering the pachytene stage (figure 2f). However despiralization of chromosomes occurred after pachytene, commencing diffuse stage (figure 2g). After the diffuse stage, diplotene stage showing secondary contraction commenced, followed by diakinesis and metaphase stages. The occurrence of diffuse stage has been reported in several plant species (Sybenga 1992), and this may be of complete type in which the whole chromosomes decondense, or it may be partial in which some parts of the genome show decondensation. The present study showed the occurrence of partial diffuse stage in the *Bromus* species studied.

Various reasons have been suggested for the occurrence of the diffuse stage, including (i) high synthetic activity, analogous to the lampbrush stage in amphibian oocytes; (ii) shedding of the lateral elements in the synaptonemal complex; (iii) post pachytene elimination or modification of histone proteins, and (iv) meiotic arrest to withstand the adverse environmental conditions (Sheidai and Inamdar 1991). Since *Bromus* species grow in various regions of Iran facing adverse environmental conditions, the occurrence of diffuse stage may be an adaptation to such conditions.

Cytomixis

Chromatin/chromosome migration occurred in different directions from early prophase to telophase-II in almost all *Bromus* species studied (figure 2a). Several metaphase/diakinesis cells in these species possessed extra or missing chromosomes showing aneuploid condition (figure 2c,d). Such aneuploid cells then form aneuploid gametes/pollen grains, and several meiocytes with extra chromosomes were observed. The other result of cytomixis is the formation of cells with missing chromatin material, which leads to the formation of abnormal tetrads, and infertile pollen grains, which was also observed in the *Bromus* species studied.

Migration of chromatin material among the adjacent meiocytes occurs through cytoplasmic connections originated from the pre-existing system of plasmodesmata formed within the tissues of the anther. The plasmodesmata become completely obstructed by the deposition of callose, but in some cases they still persist during meiosis and increase in size forming conspicuous inter-meiocytic connections or cytomictic channels that permit the transfer of chromosomes. Chromosome migration may also occur through cell wall dissolution among the neighboring meiocytes and forming syncyte (Falistocco *et al*. 1995). Cytomixis is not considered to be of great evolutionary importance, but it may lead to production of aneuploid plants (Sheidai *et al*. 1993), or result in the production of unreduced gametes, as reported in several grass species (Falistocco *et al*. 1995). Unreduced gamete formation is of evolutionary importance as it can lead to the production of plants with higher ploidy levels.

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