Cytomixis and unreduced pollen grain formation in six *Hordeum* species

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Abstract

Meiotic analysis of 11 populations of 6 *Hordeum* species showed normal chromosome pairing and chromosomes segregation during anaphase in most of the anaphase and telophase cells, except few cases of laggard chromosomes, chromosome stickiness and tripolar formation. Cytomixis and chromosome migration occurred in different directions

from early prophase to telophase-II in some of the species and populations studied leading to the formation of aneuploid cells and also formation of the meiocytes having double the normal chromosome number. Such unreduced meiocytes formed unreduced pollen grains. Two to five percent of the pollens formed were bigger in size and could be considered potential unreduced pollen grains. Other meiotic abnormalities caused some degree of pollen sterility.

Keywords: Cytomixis, *Hordeum*, unreduced pollen grains.

Introduction

The genus *Hordeum* L. (Pooideae) contains about 30 annual or perennial species, which usually grow in open weedy or sandy places, but mostly in dry soils (Bothmer et al., 1991; Shewry, 1992; Watson and Dallwitz, 1992), including weedy species like *H. jubatum*, *H. leporinum*, *H. marinum*, *H. murinum* and important grain crop species like Barley (*H. vulgare*). Diploid ($2n = 2x = 14$), tetraploid ($2n = 4x = 28$) and hexaploid ($2n = 6x = 42$) species are known in the genus (Bothmer et al. 1995), with large chromosomes and haploid nuclear DNA content of 5.4–5.6 pg and mean diploid 2c DNA value of 11 pg (Watson and Dallwitz, 1992).

Cytological studies including meiotic behavior of interspecific hybrids (Bothmer 1979, Bothmer et al., 1986a, 1986b, 1987; Zhang et al., 1990; Jahan et al., 1992, Bothmer et al., 1995; Bao-Rong, 1997; Sheidai et al., 2007), show the existence of four basic genomes labeled as H, I, X and Y in the genus. *H. vulgare* (2x) and *H. bulbosum* (2x,

4x), have the I genome, *H. marinum* (2x, 4x) and *H. murinum* (2x, 4x, 6x) have X and Y genomes respectively and the rest of diploid species have different forms of H genome.

Hordeum species grow in the north, north-west and south-west of Iran and are considered as important forage plants of the country. Parsa (1950), Mobayyen (1981) and Bor (1970), reported the occurrence of 11 *Hordeum* species in Iran; however the species recognized by these authors varies. Recently, Sahebi et al. (2004) carried out systematic review of the genus in Iran. Our previous cytogenetic study of the genus contained karyotype and chromosome pairing analysis of some species (Sheidai & Rashid 2007), while the present study considers the occurrence of cytomixis and cytological mechanisms of unreduced pollen grain formation in *Hordeum* species.

Material and methods

Hordeum species studied are 1- *H. marinum* subsp. *marinum* Hudson, 2- *H. glaucum* Steud., 3- *H. leporinum* Link., from the sect. *Hordeastrum* Döll., 4- *H. bulbosum* L., from the sect. *Bulbohordeum* Nevski, 5- *H. spontaneum* C. Koch, and 6- *H. distichon* L., from the sect. *Crithe* Döll. Nomenclature used is based on Flora Iranica (Bor 1970). Voucher specimens are deposited in Herbarium of Shahid Beheshti University (HSBU). Young flower buds were collected from 10 randomly selected plants of each species/population and fixed in Farmer fixative i.e. glacial acetic acid:ethanol (1:3) for 24 hrs. Flower buds were washed and preserved in 70% ethanol at 4°C until used (Sheidai and Rashid, 2007). Cytological preparations used squash technique and 2% aceto-orcein (Sheidai and Rashid, 2007). Fifty to one hundred meiocytes were analyzed for chiasma frequency and

distribution at diakinesis and metaphase, and 500 meiocytes were analysed for chromosome segregation during the anaphase I and telophase I.

Results

Meiotic analysis of *Hordeum* species and populations studied showed that the chromosomes mainly formed bivalents at metaphase I. Chromosomes segregation during anaphase was normal in most of the anaphase and telophase cells in all the species studied, except few cases of laggard chromosomes formation and chromosome stickiness observed (Table 1, Fig. 1. A). Laggard chromosomes led to formation of micronuclei in this species. An interesting meiotic abnormality observed was failure of anaphase-I and lack of proper chromosome movement leading to the formation of tripolar cells in 3 species of *H. leporinum*, *H. bulbosum* and *H. spontaneum* (Fig 1. E-G).

Cytomixis and chromosome migration occurred in different directions from early prophase to telophase-II in some of the species and populations studied (Table 1, Fig. 1, B-D). In some cases one or few chromosomes were migrated into the neighboring meiocyte leading to the formation of aneuploid cells (Fig. 1, J & K). In some of the meiocytes, syncyte formation and migration of the whole chromosomes led to the formation of the meiocytes having double the normal chromosome number (Fig. 1, I & M). Such unreduced meiocytes may result in the formation of unreduced pollen grains (Fig. 1. N-P), as observed in *Hordeum* species studied (discussed in the following paragraphs).

The occurrence of large pollen grains (possibly 2n pollen grains) was observed along with smaller (normal) pollen grains in populations of *H. spontaneum*, *H. bulbosum*

and *H. glaucum*, which also showed the occurrence of cytomixis (Fig. 1, N-P). The large pollen grains comprised about 2% of pollen grains in both species.

The mean diameter of normal (reduced) pollen grains was 42 µm in Vanak village population of *H. spontaneum* while, the mean diameter of unreduced pollen grains was 82 µm. The same values were 39 and 78 µm in Karaj population of the same species. Similarly the mean size of reduced and unreduced pollen grains were 36 & 70 µm in Firoozkooh population of *H. bulbosum* and 32 & 74 µm in Karaj population of *H. glaucum* respectively. The size of these two types of pollen grains differed significantly as indicated by t-test analysis ($p < 0.001$).

Pearson coefficient of correlation determined showed significant positive correlation between unreduced pollen grain formation and the occurrence of cytomixis ($r = 0.72$, $p < 0.01$). A positive significant correlation was obtained between chromosomes stickiness and cytomixis too ($r = 0.73$, $p < 0.01$). Pollen fertility showed negative correlation with chromosomes stickiness, laggard chromosomes, cytomixis and unreduced pollen grain formation, which was significant for the last one only ($r = -0.83$, $p < 0.01$).

Discussion

Migration of chromatin material or chromosomes among the 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 as it results in aneuploidy and pollen sterility. *Hordeum* species and populations with the occurrence of cytomixis also showed 4% pollen infertility. However

cytomixis may lead to production of aneuploid plants with certain morphological characteristics (Sheidai et al., 1993) or produce unreduced pollen grains as reported in several grass species including *Dactylis* (Falistocco et al., 1995) and *Aegilops* (Sheidai et al., 1999), *Aleopecurus* and *Catbrosa* (Sheidai et al., 2009). Unreduced pollen grains formation is of evolutionary importance leading to the production of plants with higher ploidy level, the phenomenon observed in *Hordeum* species studied.

Results of Pearson coefficient of correlation determined among cytogenetic abnormalities observed indicates the greater role-played by cytomixis in induction of unreduced pollen grains in *Hordeum* species studied, followed by tripolar cell formation and chromosome stickiness. More over these results indicate that all meiotic abnormalities mentioned may cause pollen sterility of the plants showing them.

The presence of giant pollen grains has been used as an indication of the production of 2n pollen. A numerically unreduced diploid or 2n is a meiotic product bearing the sporophytic rather than the gametophytic chromosome number. Such gametes result from abnormalities during either microsporogenesis (2n spores) or megasporogenesis (2n spores, Villeux, 1985).

Unreduced pollen grains are known to produce individuals with higher ploidy level through a process known as sexual polyploidization (Falistocco et al., 1995), which has been considered as the 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 of the meiocytes during the sporogenesis (Villeux, 1985). Detailed

cytological study of *Hordeum* species showed that anaphase failure and tripolar cell formation as well as cytomixis might be considered as the possible mechanisms of unreduced pollen grain formation.

The occurrence of cytomixis has been reported in *H. vulgare* leading to meiotic abnormalities and pollen sterility and the occurrence of unreduced pollen grains has been noticed in crosses between *H. chilense* X *Triticum*, (Haroun, 1996; Martin et al., 1980, 1998), therefore this is the first report on the occurrence of cytomixis and unreduced pollen grain formation in natural populations of other *Hordeum* species.

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Table 1. Meiotic abnormalities in *Hordeum* species studied. (all data in %).

Species	Locality	cytomixis	Laggard	Stickiness	Unreduced pollen	Pollen fertility
H. spontaneum	Evin	0.0	0.0	10.0	0.0	98.8
H. spontaneum	Vanak village	0.0	0.0	6.0	4.6	97.6
H. spontaneum	Karaj	15.0	3.0	18.0	5.4	98.0
H. spontaneum	Touchal	0.0	0.0	20.0	0.0	98.4
H. distichon	Darabad	0.0	0.0	7.0	0.0	99.6
H. bulbosum	Darake	8.0	2.0	25.0	0.0	98.8
H. bulbosum	Firrozkooh	6.0	3.0	22.0	5.0	98.6
H. leporinum	Evin	3.0	0.0	16.0	0.0	99.4
H. leporinum	Joybar	1.0	0.0	4.0	0.0	99.4
H. glaucum	Karaj	15.0	0.0	34.0	8.0	97.4
H. marinum ssp. *gusoneanum*	Joybar	2.0	1.0	3.0	0.0	99.4

Fig. 1. Representative meiotic cells in *Hordeum* species studied.

A = Anaphase-I cell showing laggard chromosome in *H. bulbosum*.
B & C = Prophase cell showing extra-chromosomes due to cytomixis in *H. bulbosum* and *H. glaucum* respectively.
D = Chromosome migration between adjacent meiocytes in *H. glaucum*.
E = Anaphase-II failure in *H. leporinum*.
F & G = Tripolar cells in *H. bulbosum* and *H. spontaneum* respectively.
H & I = Meiocytes showing normal (n = 7) and unreduced (n = 14) chromosome numbers in *H. glaucum* respectively.
J = Meiocyte showing aneuploidy (n = 11) in *H. bulbosum*.
K = Meiocyte showing aneuploidy (n = 28) in *H. leporinum*.
L & M = Meiocytes showing normal (n = 7) and unreduced (n = 14) chromosome numbers in *H. glaucum* respectively.
N-P = Unreduced pollen grains (bigger pollens) in *H. bulbosum*, *H. spontaneum* and *H. glaucum* respectively.

Scale bar = 10 µm