Society for Advancement of Wheat Research
ICAR-Indian Institute of Wheat & Barley Research
Karnal - 132 001, India

Journal of Wheat Research
7(2):60-63
Homepage: http://epubs.icar.org.in/ejournal/index.php/JWR

Short Communication

Meiotic abnormalities in cultivated and synthetic hexaploid wheat and their hybrids

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Article history: Received: 05 January, 2014; Revised : 25 February, 2015; Accepted: 10 December, 2015

Citation: Pachauri P and VS Kandalkar. 2015. Meiotic abnormalities in cultivated and synthetic hexaploid wheat and their hybrids. Journal of Wheat Research 7(2):60-63

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Wheat is one of the most important cereal crops in the world and is the best known plant from a cytogenetic point of view (Ecochard, 1961). During meiosis, different types of cytological irregularities can lead to 2n gametes with variable genetic composition (Matsuoka and Nasuda, 2004). Several authors have stated that meiosis is the most sensitive stage in the life cycle of crop plants, and is influenced by both genetic and environmental factors (Ahmad *et al.,* 1984; Na- muco and O'Toole 1986; Saini 1997; Porch and Jahn 2001; Erickson and Markhart 2002). Synthetic hexaploid wheat produced by crossing *Triticum turgidum* L. spp (2n=4x=28, AB genomes) with *A. tauschii* Coss. (2n=2x=14, D genome), is considered a promising source of exotic alleles for introgression into wheat (Mujeeb-Kazi *et al.,* 2008). Because hexaploid wheat has no close relative species at the hexaploid ploidy level, the synthetic hexaploid wheat can be used as an intermediary for transferring the desired traits from the wild ancestor to cultivated wheat (Del Blanco *et al.*, 2001). Meiosis is an event of high evolutionary stability which ceases in a reduction of chromosome number. Meiosis, as a highly coherent integrated process, involves 3 major tasks of co-orientation of homologous chromosomes into the opposite cell poles, reduction of chromosome number and recombination (Cnudde and Gerats 2005). The most common abnormalities reported in the *Gramineae* family included precocious chromosome migration to the poles and laggards in metaphases and micronuclei in tetrads (Mendes-Bonata *et al.,* 2006). Present investigation was conducted to identify intra- and interspecific variations of meiotic chromosome behavior of pollen mother cells using 10 wheat genotypes belonging to the 3 ploidy levels of diploid, tetraploid and hexaploid or synthetic wheats with their 4 interspecific crosses.

Wheat genotypes comprised varieties of *Triticum dicoccum* 'DDK-1038', *T. aestivum* 'LOK-1', 'RD 1336' and 'DL-788-2', and *T. durum* 'HI 8498' and Synthetic lines, Synthetic-18, 11, 19 and 22 along with 4 interspecific crosses 'Synthetic-18 X RD 1336', 'Synthetic-19 X HI 8498', 'Synthetic-22 x DL-788-2' and 'Synthetic-21 x DDK-1038'. Thirteen genotypes were sown by dibbling in single row plot of size 20 cm X 2.5 m in fully irrigated condition on 19 November, 2013. Immature spikes were collected at the early to mid-booting stage and fixed in Carnoy's solution (ethanol–chloroform–acetic acid in a 60:30:10 proportion) for 24h. Florets were then washed and preserved in 70% ethanol at 4°C until used. Pollen mother cells (PMCs) were observed under microscope after staining with acetocarmine. Chromosome images were recorded by digital camera. Normal tetrads were considered as those with four equal-sized cells. Meiotic abnormalities were recorded such as laggards, chromosome stickiness / bridge, ring bivalent, precocious chromosome migration and micronuclei per cell at different meiotic stages. About 100 PMCs at different meiotic phases were counted for laggards, chromosome stickiness / bridge, ring bivalent, precocious chromosome migration and micronuclei per cell and the frequency of cells showing at least one symptom of irregularity was used for statistical analysis.

Meiotic abnormality in synthetic wheat and their interspecific crosses with cultivated species explained applicability of synthetic lines to introduce desired segregates of new characteristics. Results of meiotic abnormality are discussed under various types of abnormality observed in the synthetic wheat and their interspecific crosses.

Ring bivalents: The frequency of ring bivalents at metaphase I ranged from 38% (DDK-1038) to 76% (HI-8498). SYNTHETIC-18 possessed 75% ring bivalents followed by 73% (SYNTHETIC-11), 71% (RD-1336), 70% (SYNTHETIC-18 X RD-1336), 69% (DL-788-2), 65% (SYNTHETIC-21 X DDK-1038), 63% (SYNTHETIC-19

X HI-8498), 60% (SYNTHETIC-22 x DL-788-2), 55% (LOK-1), 54% (SYNTHETIC-19), 50% (SYNTHETIC-22) and 38% (*T. dicoccum* var. DDK-1038) (table 1; Fig1: k, l). The similar results of ring bivalents were also reported in synthetic hexaploid wheat and tetraploid cultivated genotypes at diakinesis or metaphase-I accessions by

Table1. Meiotic abnormalities in wheat varieties of different species and their interspecific crosses

Species	Total cells	Precocious cells	Precocious chromosome migration (%)	Total cells	Chromosome Stickiness	Chromosome stickiness (%)	Total cells	Laggard Chromosome	Laggard chromosome (%)	Total cells	Micronuclei	Micronuclei (%)	Total cells	Ring bivalent	Ring bivalent (%)
SYNTHETIC-19 X HI 8498	62	1	1.61	64	1	1.56	50	1	2.00	91	2	2.20	22	14	64
SYNTHETIC-18 X RD-1336	86	2	2.33	84	2	2.38	81	3	3.70	90	1	1.11	20	14	70
SYNTHETIC-22 x DL-788-2	61	1	1.64	50	1	2.00	85	2	2.35	103	2	1.94	20	12	60
SYNTHETIC-21 x DDK-1038	72	1	1.39	0	0	0.00	80	2	2.50	101	2	1.98	20	13	65
DDK-1038	96	1	1.04	67	1	1.49	0	0	0.00	00	0	0.00	21	8	38
LOK-1	102	1	0.98	98	1	1.02	0	0	0.00	00	0	0.00	20	11	55
RD-1336	100	1	1.00	95	1	1.05	0	0	0.00	00	0	0.00	21	15	71
DL-788-2	99	2	2.02	97	1	1.03	0	0	0.00	00	0	0.00	23	16	70
HI-8498	98	1	1.02	90	1	1.11	0	0	0.00	00	0	0.00	21	16	76
SYNTHETIC-11	95	1	1.05	0	0	0.00	55	1	1.82	106	1	0.94	23	17	74
SYNTHETIC-18	85	2	2.35	0	0	0.00	84	1	1.19	95	1	1.05	24	18	75
SYNTHETIC-19	63	1	1.59	68	1	1.47	0	0	0.00	98	2	2.04	22	12	55
SYNTHETIC-22	65	1	1.54	52	1	1.92	85	2	2.35	102	2	1.96	20	10	50

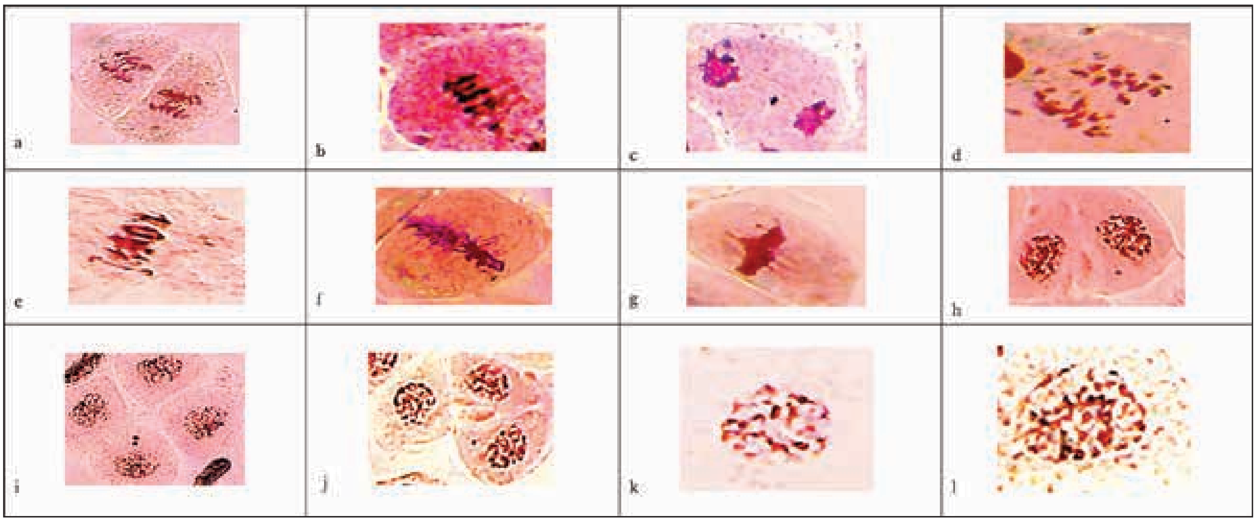

Fig.1. Meiotic chromosome behavior of studied wheat genotypes: Chromosome bridge at late metaphase II (a) in Synthetic-22; Precocious chromosome migration at metaphase stage (b) in 'SYNTHETIC-18 X RD-1336', (e) in LOK-1 and (f) in DDK-1038; Laggard chromosome at telophase stage (c) and anaphase stage (d) in 'SYNTHETIC-18 X RD-1336'; Chromosome stickiness at metaphase stage (g) in DDK-1038; Micronuclei at telophase stage (h), at triad cell (j) and micronuclei at tetrad cell (i) in 'SYNTHETIC-19 X HI-8498'; Ring bivalent at diakinesis stage (k) in 'SYNTHETIC-22 X DL-788-2' and (l) in 'SYNTHETIC-18 X RD 1336'

Arabbeigi *et al.,* (2010). Ring bivalents were observed more in parents compared to their interspecific crosses. Three major meiotic processes, chromosome pairing, synapsis and crossing over, are involved in the formation of bivalents (Bass *et al.*, 2000). Homologous chromosomes previously distributed throughout the nucleus need to approach and recognize each other to enter into intimate contact and form bivalents (Maestra *et al.,* 2002). A high chiasma frequency at meiosis and the formation of at least 1 chiasma per arm caused the formation of ring bivalents in the most metaphase-I preparations (Moss and Murray 1990).

Laggard Chromosome: The frequency of cells in the anaphase and telophase stages possessed higher laggard chromosomes in interspecific crosses 3.7% (SYNTHETIC-18 X RD-1336) followed by 2.50% (SYNTHETIC-21 X DDK-1038), 2.35% (SYNTHETIC-22 X DL-788-2) and 2.00% (SYNTHETIC-19 X HI-8498) as compared to their parents. Among the parents, (SYNTHETIC-22) recorded highest laggard chromosomes 2.35% followed by 1.82% (SYNTHETIC-11) and 1.19% (SYNTHETIC-18) (table 1; Fig1: c, d). Similar findings of laggard chromosome also reported in synthetic wheat accessions by (Arabbeigi *et al.,* 2010). Interspecific crosses possessed comparatively highest amount of laggard chromosomes compared to their parents. Chiasma are responsible for the maintenance of bivalents which permit normal chromosome segregation (Bione *et al.,* 2000), therefore, any failure in chiasma formation and /or the occurrence of any crossing-over may lead to laggard chromosomes.

Precocious chromosome migration: At metaphase I and II stages, parent 'SYNTHETIC-18' recorded highest precocious chromosome migration of 2.35% compared to interspecific crosses. Similarly, interspecific crosses showed higher precocious chromosome migration of 2.33% (SYNTHETIC-18 X RD-1336) followed by 1.64% (SYNTHETIC-22 X DL-788-2), 1.61% (SYNTHETIC-19 X HI 8498) and 1.39% (SYNTHETIC-21 X DDK-1038). Parents DL-788-2 recorded highest precocious chromosome migration of 2.02% followed by 1.59% (SYNTHETIC-19), 1.54% (SYNTHETIC-22), 1.05% (SYNTHETIC-11), 1.04% (DDK 1038), 1.02% (HI-8498), 1.00% (RD-1336) and 0.98% (LOK 1) (Fig1: b, e, f). Arabbeigi *et al.,* (2010) also reported similar precocious chromosome migration in synthetic wheat. Precocious migration of chromosome to the poles is a very common abnormality among plants (Pagliarini and Pereira 1992, Consolaro *et al.,* 1996). Precocious chromosome migration to the poles may have resulted from univalent chromosomes at the end of prophase-I or precocious chiasma terminalization in diakinesis or metaphase-I. Univalents may originate in the absence of crossing-over in pachytene or from synaptic mutants (Bione et *al.,* 2000).

Chromosome stickiness / bridge: At the anaphase and telophase stages, interspecific crosses recorded higher chromosome stickiness in 'SYNTHETIC-18 X RD-1336' (2.38%) followed by 'SYNTHETIC-22 X DL-788-2' (2.00%) and 'SYNTHETIC-19 X HI-8498' (1.56%) compared to most of the parents. Similarly, parents also recorded chromosome stickiness / bridge in SYNTHETIC-22 (1.92%) followed by DDK-1038 (1.49%), SYNTHETIC-19 (1.47%), HI-8498 (1.11%), RD-1336 (1.05%), DL-788-2 (1.03%) and LOK 1 (1.02%) (table 1;Fig1: g). Chromosome stickiness were also reported in synthetic wheat accessions by Arabbeigi *et al.,* (2010). Chromosome stickiness may be caused by genetic or environmental factors such as X-rays, gamma rays, temperature, herbicides or some chemicals present in soil (Caetano- Pereira *et al.,* 1995, Nirmala and Rao 1996). Synapsis between inverted and non-inverted chromosomes is accompanied by loop formation in pachytene. The occurrence of crossing over within the inversion loop results in the formation of a dicentric bridge and an acentric fragment, as well as 2 structurally normal chromatids at anaphase I (Stoinova *et al.,* 2006).

Micronuclei / univalent: The interspecific cross 'SYNTHETIC-19 X HI-8498' recorded highest amounts of micronuclei in the tetrad cells of 2.20% compared to rest of crosses and parents (Table; Fig 1: h, i, j). Other interspecific crosses were also recorded considerable amounts of micronuclei in the tetrad cells in 'SYNTHETIC-21 X DDK-1038 (1.98%), 'SYNTHETIC-22 X DL-788-2' (1.94%) and 'SYNTHETIC-18 X RD-1336' (1.11%). Synthetic lines possessed higher amounts of micronuclei in the tetrad cells in SYNTHETIC-19 (2.04%) followed by SYNTHETIC-22 (1.96%), SYNTHETIC-18 (1.05%) and SYNTHETIC-11 (0.94%). Micronuclei was observed more at diad and tetrad cells and very few in triad cells of interspecific cross 'SYNTHETIC-19 X HI-8498'. The similar results of micronuclei / univalent were also reported earlier in synthetic wheat accessions by (Arabbeigi *et al.,* 2010). It is characteristic of univalent and laggard chromosomes that they generally lead to micronuclei formation (Koduru and Rao 1981). In telophase-I, the bivalents gave rise to micronuclei with an extremely wide variation in number and size (Pagliarini, 2000). Laggards caused genetically inactive micronuclei at telophase-II only in single tetrads. At this phase, cell partitions are properly situated, except for 1 preparation where 2 triads were observed (Stoinova *et al.*, 2006).

The Meiotic analysis of interspecific crosses of synthetic hexaploid wheat revealed that crosses 'SYNTHETIC-21 X DDK-1038', 'SYNTHETIC-19 X HI-8498', 'SYNTHETIC-18 X RD-1336', DDK-1038 and 'SYNTHETIC-18 X RD-1336' possessed very low percentage of ring bivalents, micronuclei, laggard chromosomes, chromosome stickiness and precocious chromosome migration, thereby, suggesting that these synthetic lines as new source of desired new exotic alleles may easily be used for introgression into wheat.

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