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CHROMOSOMAL BEHAVIOR STUDIES DURING MEIOSIS: A CROSS BETWEEN *Triticum timopheevi* and *Triticum sphaerococcum*

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Abstract- Wild species of wheat belonging to different ploidy groups namely *Triticum timopheevi* (2n=28), and *Triticum sphaerococcum* (2n=42) were crossed. Very few seeds obtained from the cross between *T. timopheevi* as female and *T. sphaerococcum* as male, which were small in size and shriveled in nature. These seeds were raised and the viable hybrids were studied for their chromosomal behavior during meiosis. They showed abnormal pairing and irregular separation thus leading to sterile pollen grains. The morphology of the hybrid was almost similar to the female parent except for the dominant character like anwness, which showed pollen parent inheritance. Abnormal tetrad formation, micronuclei formation leading to polymorphic pollen grains leading to sterility was commonly observed.

Keywords- Chromosome, Wheat, Ploidy and interspecific crosses.

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Introduction

The cultivated species of wheat can be divided into three definite groups according to their sterility relationships in inter-specific crosses [1] namely Einkorn the diploid wheat, Emmer the tetraploid wheat and Vulgare hexaploid wheat. Among the cereals, wheat was the first crop to be domesticated and constitutes as the major staple food crop for about 35% of the world population. Now the wheat productivity has reached a ceiling in yield. The amount of usable genetic variability is on decline. There have been major threats to genetic diversity in general. The different species of genus *Triticum* are diverse in their genotypes and phenotypes that are adapted to a wide range of environments and hold rich pools of genetic variation which could be utilized to improve the common wheat. The characters include resistance to pathogens, drought tolerance, winter hardiness, adaptability to poor soil and even high protein content. The diploid, tetraploid and hexaploid wild wheats share one or more genome with cultivated (*T. dicoccum, T. durum, T. aestivum*) wheat and they play a vital role in wheat improvement through transfer of beneficial genes. To improve any crop, breeders derive the desired genes from the existing gene pool available in the natural population. Since the genetic diversity within the cultivated wheat is declining there is a need to create variability which is made possible through wide crossing and on the success of the crosses attempted.

In the present study two wild species of genus *Triticum* with different ploidy levels, *viz.*, *T. timopheevi* (2n=28), and *T. sphaerococcum* (2n=42) were crossed. This study was carried out to produce viable synthetics so that desirable genes can be transferred into cultivated wheat and to study about the homology between different species. The study focused on the crossability of the species, cytogenetic behaviour of the hybrids, their pollen and spikelet fertility.

Materials and Methods

T. timopheevi, used as female parent is a tetraploid wild species with genome of AAGG is one of the best source for the cytoplasmic male sterility. This species is a rich source of stem rust, leaf rust resistance genes and has a profuse tillering character.

T. sphaerococcum, used as male parent is a hexaploid wild species with genome of AABBDD is one of the sources for disease resistances like powdery mildew and free thresh ability.

Emasculation and pollination

At the time of flowering, the spikes ready to flower on the next day were selected for emasculation. Hand emasculation was carried out between 9 am to 12 am in *T. timopheevi*, which have been used as female parents. In the next day morning, anthers ready to dehisce were collected from *T. sphaerococcum* and dusted on the emasculated spike. Pollination was carried out from 8.30 to 10.30 am, and the pollinated spike was covered with paper bag. Pollination was done continuously for two days. Seeds from crossed spikes were collected after the crossed seeds were germinated in petripates and after germination; they were transplanted in field on 15th day.

Cytological studies

i) Meiosis

For the study of meiosis, young spikes of appropriate size were fixed in Carnoy's fluid (absolute alcohol and glacial acetic acid in the proportion of 3:1) between 9 am and l0am, when there was bright sunlight. The material was kept in the fixative for 24 hours at 4°C and then transferred to 70 per cent alcohol and stored in the refrigerator until taken out for use. The meiosis was studied in temporary smears of pollen mother cells using 1 per cent acetocarmine stain. The fixed spikes were washed with distilled water to remove the trace of alcohol. Anthers were dissected out and placed on a clean side. A drop of acetocarmine was added and the anther was smashed using needle. The anther walls and debris were removed and a cover slip was placed on the squash such that no air bubble was formed. The slide was warmed over a flame for 2 seconds and pressed to have a good spread of PMCs. The slides were examined under microscope.

The PMCs with good spread, which were in different stages such as diakinesis, metaphase, anaphase and tetrad, were studied for chromosome association and chromosome behaviour. The chromosome associations in diakinesis were counted. More than hundred well spread cells were counted and the mean was calculated. Similar observations were taken at other stages like anaphase I and tetrad to study the chromosome behaviour in the inter-specific hybrids. Minimum hundred cells were counted in each stage.

ii) Pollen fertility studies

Anthers from different hybrids and parents were collected and placed on slide, over a drop of 1% acetocarmine mixed with glycerol (1:1) and tapped for the release of pollen grains. A cover slip was placed after removing the debris. It was left as such for 3 hours for the pollen grains to get stained. After that, the pollen grains were observed under the microscope for their stain ability. All the fully stained pollen grains were recorded as fertile and those partially stained or fully unstained or shrunken were counted as sterile.

Results and Discussion

Inter-specific hybridization

The cross between *T. timopheevi* with *T. sphaerococcum* yielded 39 seeds with a success of 26.17%. Only 11 out of 39 seeds germinated (28%) for the cross. Viability of the hybrids was 28.2% for the cross with *T. durum* and 12.3% with *T. aestivum*. The seeds obtained were very small in size and shrivelled in nature.

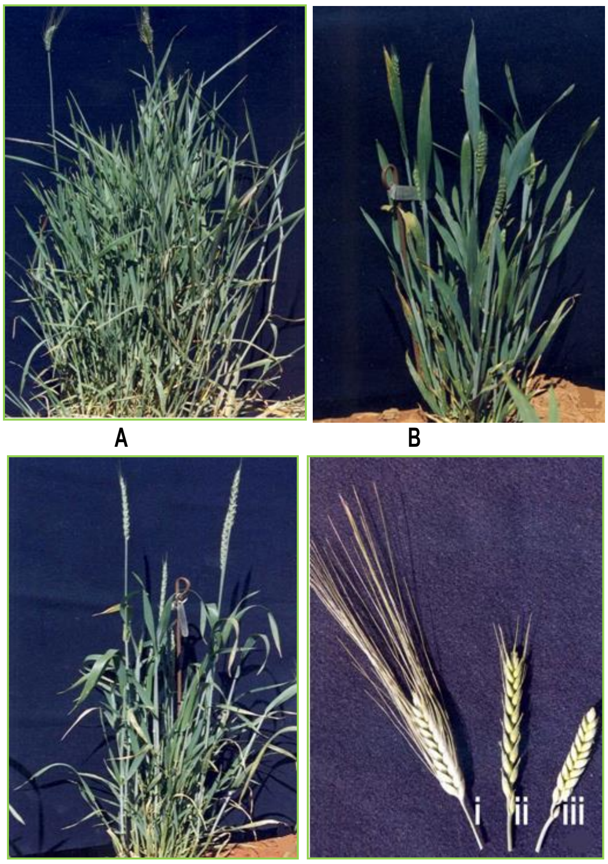


Fig-1 (A) *T. timopheevi* (B)*T. sphaerococcum* (C)*Interspecific hybrid* (D) *Panicles:* i) *T. timopheevi ii) hybrid iii) T. sphaerococcum*

Morphology of interspecific hybrids

All the hybrids of the cross were bushy with an average height ranging from 82 to 87 cm. Tillering was medium resembling that of the male parent. The leaves of hybrids were pubescent and started to produce spikes from day 74. The average panicle length varied from 6.2 to 6.5 cm, which was longer than both the parents. The awns were absent in the hybrids like that of male parent. The peduncle was more similar to the female parent and longer than the male [Table-1] & [Figure-1]. Hybrids produced white coloured anthers with sterile pollen. They were fully sterile and set no seeds on selfing.

Meiotic behaviour of inter-specific hybrids

Hybrid of *T. timopheevi* with *T. sphaerococcum* were of 2n=35. The

Table-1 *Morphological description of interspecific hybrids between T. timopheevi x T. sphaerococcum*

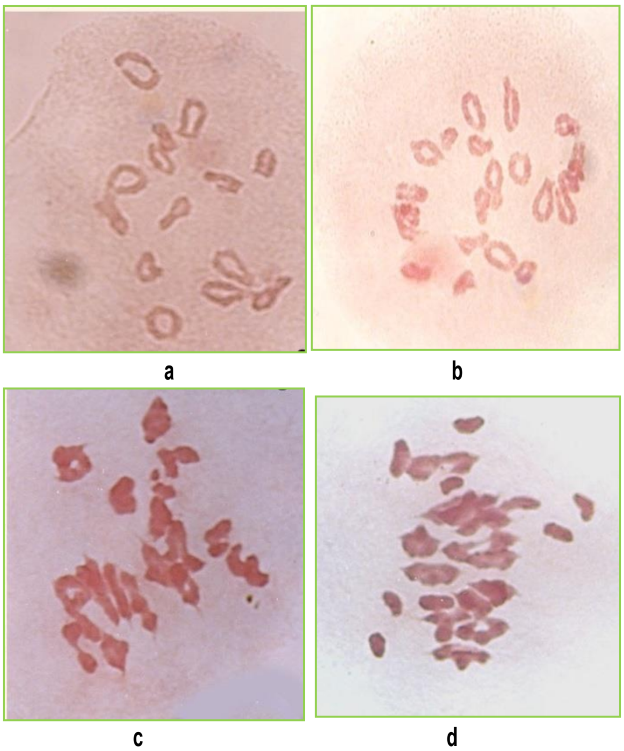
S.No	Characters	*T. timopheevi*	F_1 between *T. sphaerococcum* and *T. timopheevi*	*T. sphaerococcum*
1.	Plant height (cm)	112 ±5	82 ± 5	76 ±5
2.	Tillering	Profuse	Medium	Medium
3.	Leaf colour	Dark green	Green	Green
4.	Days to heading	110	74	65
5.	Presence of awns	Present	Absent	Absent
6.	Panicle length(cm)	6.1 ±0.5	6.4 ±0.5	5.9 ±0.5

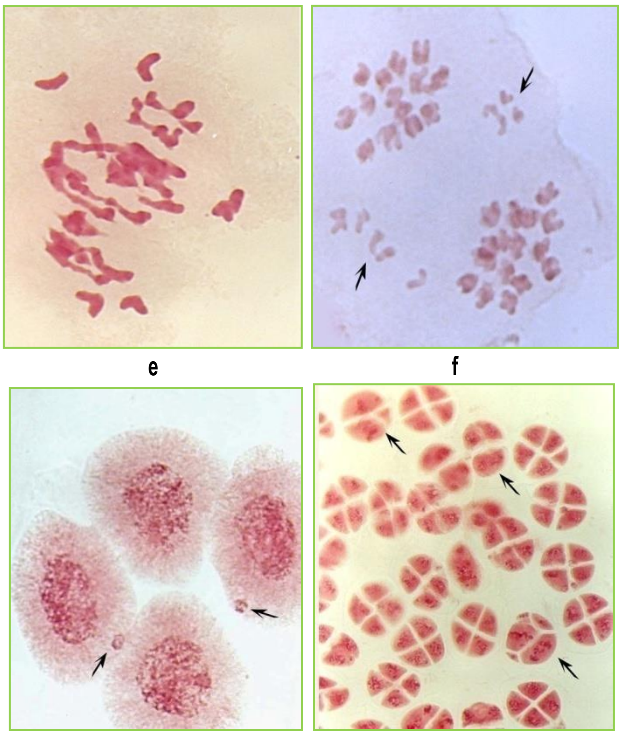
microsporocyte cytology of these hybrids showed univalents, trivalents, and quadrivalents at diakinesis / metaphase I [Table-2]; [Figure-2]. The average number of univalents ranged from 5- 11, bivalents 4- 11, trivalents from 1- 5 and quadrivalents 0 to 1. Two pairs of precocious chromosomes were found in anaphase I. Abnormal anaphase separation and bridge formation were common and many laggards were seen in the hybrid [Table-3]. A preponderance of two and four laggards in the interspecific hybrid was seen. Abnormal tetrad formation and frequent formation of dyad, triad, pentad, and hexad were observed. Formation of micronuclei was also found to be common. The shape of the pollen grains showed polymorphism and the pollen grains were completely sterile.

Table-2 *Meiotic chromosome association at Diakinesis/ Metaphase I in different interspecific hybrid*

Interspecific hybrid	Chromosome association at diakinesis / metaphase I*				No. of PMC's observed	Most frequent association
	I	II	III	IV		
T.timopheevi (2n=28) x *T. sphearococcum* (2n=42)	5-12 (11.36)	4-11 (4.48)	1-5 (4.49)	0-1 (0.60)	112	11I+4II+4III +1IV

*Figures in the parenthesis are weighted mean of the chromosome association





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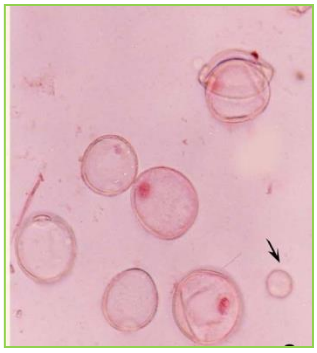

i

Fig-2 Cytological behaviour of interspecific hybrids of *T. timopheevi x T. sphaerococcum,* Arrow points indicate locations of abnormalities

- a) **Diakinesis with 14 II of *T. timopheevi***
- b) **Diakinesis with 21 II of *T. sphaerococcum***

Figures (c-i) show meiotic behaviour of microsporocytes in the hybrids

- c) **diakinesis (with univalents, 2I + 13II + 1III + 1IV, x600)**
- d) **metaphase (non-orientation of univalents, 4I + 12II + 1III + 1IV, x600)**
- e) **early anaphase (x600)**
- f) **late anaphase (x600) with laggards**
- g) **tetrads with micronuclei (x400)**
- h) **sporad formation -dyad, triad, tetrad, and pentad (x 400)sterile pollengrains (x400)**

Table-3 *Chromosome behaviour at Anaphase I in interspecific hybrid*

Cross combination	No of laggard chromosomes observed								Total no. of cells	No. of bridges
	1	2	3	4	5	6	11	normal		
T. timopheevi x *T. spheococcum*	7	13	36	20	33	4	4	3	120	0-1

Interspecific hybrid had many of the morphological characters similar to that of female parent except for the awnness which was dominant and was inherited from the pollen parents. The quantitative traits such as plant height and heading date were intermediate but skewed towards the maternal parent. It has been reported in wheat that substitution of nuclear components into the background of alien cytoplasm, caused morphologically inferior phenotypes in terms of plant height, vigour, biomass, changes in ear traits, and prolongation of heading time [2]. In most of the earlier studies, the higher ploidy have been used as male for easy crossability. In this study, we have deliberately used the lower ploidy as female to incorporate the effect of alien cytoplasm into the hybrids since the cytoplasm of T. timopheevi carries cytoplasmic male sterility.

The hybrid diakinesis studies showed the existence of univalents, bivalents, trivalents and tetravelents. The average number of univalents ranged from 5- 11, bivalents 4- 11, trivalents from 1- 5 and quadrivalents 0 to 1. *T. timopheevi* is distinguished from other species by the presence of G genome inherited from *Aegilops speltoides* [3]. Furthermore, a founder translocation involving chromosomes 6A, 1G, and 4G distinguishes *T. timopheevi* from cultivated tetraploids [4]. Pairing was abnormal, existence of univalents showed that the G genome usually did not pair with any of its counterpart. The cultivated and wild forms of *T. timopheevi* (AtAtGG) are known to provide disease resistance genes [5, 6].

Conclusion

T. sphaerococcum (AABBDD) is one of the sources for disease resistance gene against powdery mildew [7]. The formation of trivalents and tetravalents showed that there may be homology between G genome of *T. timopheevi* and B and D genome of *T. sphaerococcum* as reported earlier by [8]. Furthermore, the observations led to conclude that the G genome could be fostering multivalent formation among B and D genomes in other crosses because such types of pairings were normally absent in hexaploid wheat in the absence of the G genome. These meiotic anomalies resulted in the formation of anomalous pollen precursor cells, resulting in polymorphic sterile pollen grains. These genomes, though homeological, are incorporated with specific homeologous pairing supressors similar to *Ph1* allele on the long arm of chromosome 5B [9]. The presence of precocious chromosomes of about 1-2 pairs possibly points to this evidence. These laggards were leading to the formation of micronuclei affecting the pollen fertility. Thus the pollen grains showed polymorphism and sterility due to the abnormal division.

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