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Cytological analysis of distant hybrids of the soft wheat

Abstract

Interspecific hybrids with different number of chromosomes in meiosis were obtained during the analysis of 4 interspecific hybrids combination BC1 (Nadezhda x *T. timophevii) Triticum (T.)* Nadezhda*;* (K-2780 x *T. timophevii*) x K-2780; (*T.dicoccum* x Kokbidai) x Kokbidai; (K-2780 x *T. timophevii*) x K-2780, derived from crosses of soft wheat varieties with wild wheat species of *T. timopheevii*, *T.dicoccum* and hexaploid *T. kiharae. From one to six* reconstructions were revealed in the karyotypes of interspecific hybrids. In accordance with the mechanisms of emergence they can be divided into pycnosis, translocations asynapsis, di-and tricentric bridges.

Key words: interspecific hybrids, cytological analysis, meiosis, chromosome.

Introduction

The study of chromosomal associations in MI meiosis in interspecific and intergeneric hybrids provides an insight of a homeology level of different genomes Triticinae and formulates the concept of homeology group of chromosomes that combine different genome chromosomes, and also creation of foreign-substituted and foreign-added lines using monosomic lines [1-3].

Despite the weakening of the conjugation of some chromosome pairs found in certain plants of different generations of hybrids and backcrosses, the general trend of meiosis stabilization in all its phases was not disordered [4].

While working with interspecific hybrids, the main way to study chromosome disorders in meiosis is a cytological analysis. Analysis of meiosis gives very important information about the genetic relationship of chromosomes of studied forms, as stabilization of meiosis is indispensable to guarantee the preservation of genotypic features in plant reproduction by self-fertilization [5].

The main characteristic of the meiosis process - is the conjugation of homolog chromosomes. Homology of chromosomes is determined by kinship of their individual sites and localized genes, which attraction to each other determines the attraction of chromosomes. If significant changes occur in the chromosome and gene structure, it becomes non-homologous chromosomes, and will not form conjugated bivalents. Bivalent formation is necessary for regular chromosome segregation to the poles, providing a complete haploid set of chromosomes in the gametes and recombination of genes located on chromosomes [6].

Materials and methods

Research objects: varieties Nadezhda, Kokbidai, Arap, sample K-2780, wild species – *T. timopheevii* Zhuk (2*n*=28, A^tA^tGG), *T. kiharae* Dorof. et Migusch. (A^tA^tGGDD)*, T. dicoccum* Shuebl (A^uA^uBB), hybrids F_1, F_2, BC1 .

Research methods: plants were fixed for cytological analysis in the mornings from 6 till 8 a.m. in fair weather and from 6 a.m. till 10 a.m. in cloudy days. Carnoy's fluid (3 parts of ethanol and 1 part of acetic acid) and Newcomer fixative (isopropanol – 6 parts, propionic acid – 3 parts, dioxane – 1 part, petroleum ether – 1 part*,* 1 part acetone) were used for fixation [7].

Fixed material was filled with fresh fixative in a day and stored in the refrigerator. After washing, the fixed material was separated from the rod of spiked flowers and stained. Acetocarmine dye used for coloring was prepared by the standard technique [8, 9].

Microsporogenesis was studied in temporal preparations pressed in 45% acetic acid, using light microscopes MBI-3, IBI-6.

During cytological studies observations of the correctness of the meiosis process in mother pollen cells (MPC) were conducted, and irregularities in metaphase 1 (MI), anaphase I (AI), anaphase 2 (AII) and in the tetrades were noted.

Disorders in meiosis were studied by the usual method Pausheva and Sears [8, 10].

Pictures of different microsporogenesis stages specific for crossing combinations analysis were photographed on the microscope Axioskop 40 (Zeiss, Germany).

Results and their discussion

A key step in determining the stabilization of karyotypes of interspecific hybrids and the nature of intergenomic chromosome substitution is the formation of viable first-generation hybrids gametes.

Multivalent associations, tetravalents, giant cells with fragments, bivalent chromosome conjugation with fragments were detected in almost all F_1 hybrid combinations derived from the crossing of tetraploid wheat species *T. timopheevii* Zhuk (2*n*=28, A^tA^tGG), *T. kiharae* Dorof. et Migusch. (A^tA^tGGDD), *T. dicoccum* Shuebl (A^uA^uBB) withsoft wheat *T. aestivum* var.*velutinum* (Fig. 1, 2).

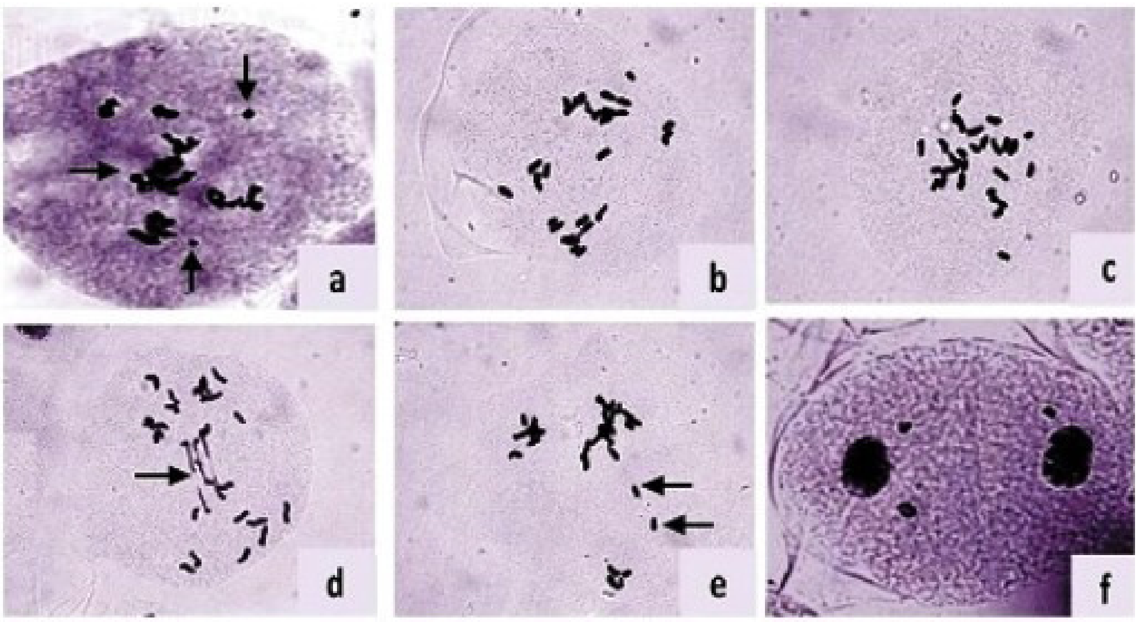

Figure 1 – Meiosis of maternal pollen cell of F_1 distant hybrids derived from the cross of the soft wheat with *T. timopheevii*. Note: a – metaphase (MI) of meiosis (chromosome pyknosis and runaway chromosome fragments are pointed by arrow); b – initial stage of pycnotic chromosome segregation to poles; c – partial chromosome conjugation in meiosis metaphase; d - AI of meiosis with tricentric bridges, pointed by arrow; e – three poles anaphase (AI) with lagging univalents; f – telophase with micronucleus.

Significant abnormalities revealed in meiosis in particular cytological analysis of F_1 hybrids from crosses of wild type *T. timopheevii* with soft wheat varieties. Basically chromosomes in metaphase and anaphase were liable to partial pyknosis. Discovered disorders in meiosis suggest the presence of foreign genetic material in a hybrid offspring. These morphological analyses confirm this assumption. To overcome the sterility of F_1 hybrids, we used mediator and backcrossing methods.

Analysis results of MPC meiosis of BC1 hybrids are shown in tables 1 and 2.

Table 1 – Average number and the variation of chromosomes disorders in M1 MPC of hybrids BC1 wheat x *T. timopheevii*

Hybrids	Number of MPC	Bivalents		Univalents	Trivalents	Tetravalents
		Closed	Open			
(Nadezhda × *T. timophevii*) × Nadezhda	75	9.32 ±0.02	4.55±0.03	12.6 ± 0.03	0.1 ±0.01	0.2 ±0.01
(K-2780 × *T. timophevii*) × K-2780	73	12.44 ±0.06	1.72 ± 0.05	9.5 ± 0.05	0.5 ±0.01	0.05 ±0.01

Availability of multivalents in meiosis MI of MPC hybrids indicates genes recombination and the possibility of translocations formation between chromosomes of ABD wheat genomes and chromosomes of genomes A^tA^tGG, A^tA^tGGDD and A^uA^uBB. Chromosomes conjugation in meiosis M1 of MPC hybrids Nadezhda x *T. timophevii* showed the presence of 13 and 14 bivalents in average per pollen mother cells in combination (Nadezhda x *T. timophevii*) x Nadezhda, and in combination (K-2780 x *T. timophevii*) x K-2780 (Table 1).

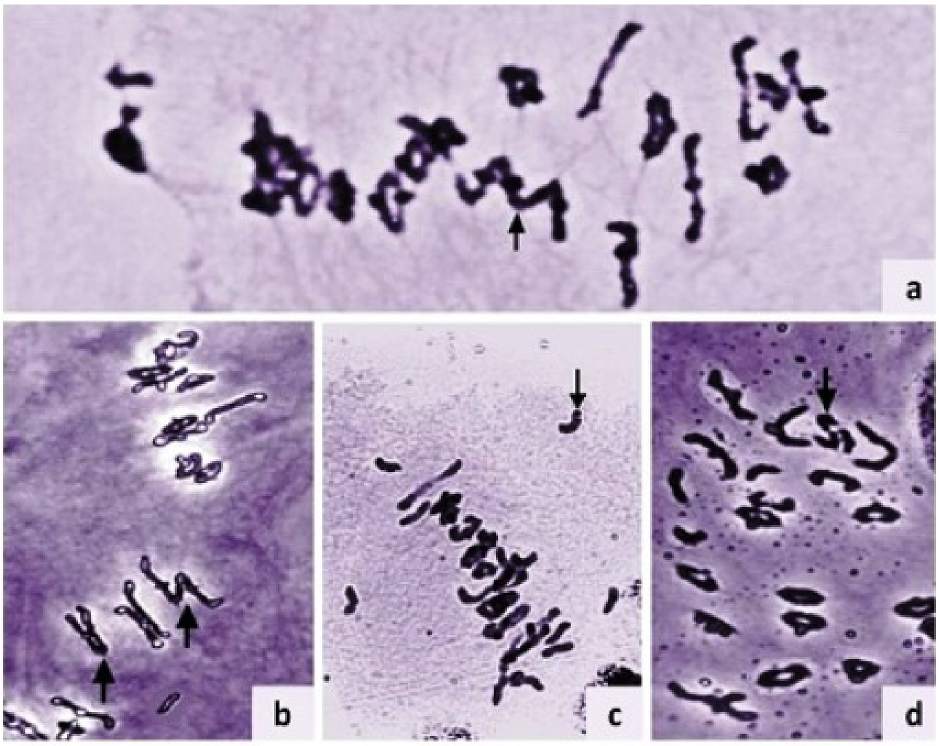

Figure 2 – Maternal pollen cell meiosis of distant BC1 hybrids derived from the cross of the soft wheat with *T. dicoccum* and *T. kiharae.* Note: a – MI (16^{II} + one hexavalent +1^{I}) BC1 with *T. kiharae*; b – MI (14^{II} + one trivalent and one quadrivalent + 1^{I} with *T. kiharae*; c – MI (18^{II}+7^{I}, one of them with the satellite, pointed by arrow) BC1 with *T. dicoccum*; d – MI (15^{II}+3^{I}) + one trivalent and three univalents, pointed by arrow.

Table 2 – *Average number and* the variation of chromosomes disorders in M1 MPC of hybrids BC1 wheat x *T. kiharae* and *T. dicoccum*

Hybrids	Cell number	Bivalents		Univalents	Trivalents	Tetravalents	2n
		Closed	Open				
(*T.dicoccum* x Kokbidai) × Kokbidai	70	1.98±0.02	17.17±0.04	1.6±0.03	0.1±0.01	-	35
(Arap × *T. kiharae*) × Arap	65	7.5±0.04	12.19±0.01	7.94±0.07	0.33±0.01	0.14±0.01	49

As can be seen on Figure 2, completed by the data in Table 2, the compatibility of the genomes of interspecific hybrids depends on the genotypes taken for hybridization of wheat varieties and the direction of the crossing. This is clearly seen in interspecific hybrids F_0. F_1 hybrids with species *T. timopheevii* and *T. kiharae* are completely sterile, and in combination with a *T. dicoccum* produce single grains. Moreover, interspecific hybrids showed high resistance to fungal diseases. However, the instability of the genome of interspecific hybrids requires backcrossing, continuous monitoring of the number of chromosomes in the hybrid offspring and identifying of stable irtrogressive lines with 42 chromosomes.

Conclusion

All F_1 hybrid plants were morphologically intermediate between the parents. Seed set in the combinations listed above was low, and the degree of seed germination was high. Grooves of hybrid seeds obtained by combinations of crossing *T. dicoccum* with soft wheat were less expressive compared with the grooves in the seed combinations F_1 of *T. timophevii* and *T. kiharae* with soft wheat. Phenotypes of F_1 and F_2 interspecific hybrids were mostly determined by signs of wild species, indicating the recombination of parental form genes, however, an intermediate inheritance of the ear phenotype was revealed in offspring hybrids of intermediaries with BC1.

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