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MEIOTIC STUDIES OF SECONDARY 42-CHROMOSOME TRITICALES[1]

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Abstract

Cytological studies were conducted to determine the relationship of chromosomal behavior to fertility and to establish the chromosomal nature of secondary triticales. Examination of number of univalent chromosomes, micronuclei in tetrads, pollen stainability, and percentage of trinucleate pollen showed a wide range of irregularity among nine secondary 42-chromosome triticales and four intertriticale hybrids. The number of lagging chromosomes varied from 0 to 8 in each variety. Irregularities during meiosis appeared to be related to differences in the time of replication of the wheat and rye chromosome. The frequency of mature (trinucleate) pollen was correlated with the regularity of meiosis and pollen stainability (I-KI), but not with the number of micronuclei in tetrads, suggesting at least some of the fertility variation in triticales is due to meiotic disturbances.

Introduction

Triticale is a synthetic cereal grain obtained by combining the genomes of wheat (*Triticum*) and rye (*Secale*). The name Triticale was coined from the prefix of *Triticum* and the suffix of *Secale*, the parental genera. In the early 1930's triticale began to be evaluated as a potentially new field crop (Briggle, 1969; Quinones, 1973).

The most promising hexaploid triticales have been obtained from intercrosses of hexaploid with hexaploid and octoploid triticales (Pissarev, 1963; Kiss, 1966) or hexaploid wheats (Nakajima and Zennyozi, 1969; Zillinsky and Borlaug, 1971). Triticales produced by the latter method are usually referred to as secondary triticales which are obtained by recombination due to hybridization and selection between two or more primary triticales or with wheats.

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The general purpose of developing new triticale is to unite the milling and baking qualities of wheat with the growth ability of rye, which can be grown on poor and dry soils. Immediate application of species triticale as a new cultivated cereal in agriculture is rather limited by two main obstacles: low fertility and shriveled grains. To overcome these problems for triticale improvement, the breeding programs were directed towards (1) producing new promising triticales, (2) crossing the triticales to the local wheat varieties, and (3) intercrossing different lines of triticales and selecting segregates with high fertility and promising agronomic characteristics in hybrid populations.

Despite the difficulties mentioned above, there are indications that triticale has a theoretical potential of producing high yield in some areas. In practice, the potential has not been realized because of limiting factors associated with reproductive system. These defects should be amenable to genetic improvement.

The primary objective of this study was to find possible relationships among chromosomal behavior, pollen development and seed set fertility in hexaploid triticale to establish a basis for the predication of the results of future crossing and to aid in the selection of parental materials for hybridization. The secondary objective was to examine the meiotic instability in improved secondary triticales.

Materials and Methods

Nine hexaploid triticales 6TA-204, 6TA-419, Armadillo-133, Armadillo-1524, Armadillo-T909, PPV-21, Bruin-46, and Bronco-90, were included for cytological study in a greenhouse experiment in the summer of 1972. The origin and parentage of the parental materials are listed in Table 1. Eight intercrosses (Table 1) among 42-chromosome triticales were investigated in the F_1 germination for meiotic characteristics. All crosses were made in a greenhouse during the spring, 1972. F_1 plants were obtained without embryo culture and grown in a greenhouse. One spike was sampled per plant from the first tiller. Samples were fixed for 24 hr in acetic acid and ethanol (1:3) and stored in 70% ethanol. Standard acetocarmine squash methods were used for meiotic chromosomal analysis.

The following cytological characteristics were observed:

(1) The number of univalent chromosomes at first metaphase;
(2) The number of lagging chromosome at first anaphase;
(3) The number of tetrads classified into 5 groups having tetrads with 0 to 4 cells with micronuclei;
(4) The percentage of stainable pollen determined by I-KI staining;
(5) The percentage of viable pollen as shown by acetocarmine stain;

Table 1. *Parental materials*

Genetic material	Chromosome number	Source
6x-triticale		
6TA-204	42	B. C. Jenkins
6TA-419	42	B. C. Jenkins
Armadillo 133	42	CIMMYT
Armadillo 1524	42	CIMMYT
Armadillo T909	42	CIMMYT
PPV-13	42	CIMMYT
PPV-21	42	CIMMYT
Bruin-46	42	CIMMYT
Bronco-90	42	CIMMYT

Eight combinations of intertriticale hybrids (Bronco-90 × 6TA-419, 6TA-204 × Arm. 133, 6TA-204 × Arm. 1524, 6TA-204 × Arm. T909, 6TA-204 × PPV-13, 6TA-204 × PPV-21, 6TA-204 × Bruin-46, 6TA-204 × Bronco-90).

ability in which mature pollen contained three normally developed nuclei.

A total of 200 pollen mother cells from each genotype were observed.

Results

Meiotic Study of 42-chromosome Triticales

In parental lines, chromosome pairing was evidently extremely erratic with very low frequency of regular bivalents per PMC (Fig. 1). The univalents may result either from the failure of the chromosomes to pair at zygotene (asynapsis) or of paired chromosomes separating at diplotene because chiasma formation did not occur (desynapsis). In triticale side-by-side associations were rare so the lack of pairing was probably asynaptic rather than desynaptic in origin. This suggests disturbances during diplotene or zygotene rather than interference with the timing of the intimacy of chiasma formation. Fig. 1B shows different time sequences of chromosome replication of wheat-rye which were grouped into two distinctive chromosome clusters (Fig. 2B, C). Multivalent associations were observed and it was not possible to be certain of the number of chromosomes involved or to make accurate counts of the number of bivalent chromosomes, thus, at metaphase only univalents and lagging anaphase chromosomes were analyzed (Table 2). Meiosis was irregular in all strains, with failure of pairing being the common feature. The frequency of lagging chromosomes differed among the cultivars and ranged from 0 to 8 in each cultivar. It was not possible to determine whether the rye or wheat

Table 2. *Meiotic characteristics of hexaploid triticale*

Cultivar	No. of lagging chromosomes at MI-AI, % of cells									Tetrads, percent cells with micronuclei in quartet					Pollen, %		
	0	1	2	3	4	5	6	7	8	0/4	1/4	2/4	3/4	4/4	Without micro-nuclei	Stained	With 3 nuclei
Triticale																	
6TA-204	26	14	10	10	10	18	4	4	4	36	18	23	18	5	66	94	91
6TA-419	38	5	16	9	13	8	3	6	2	61	7	19	7	6	77	94	92
Arm. 133	6	15	19	18	18	8	5	6	5	52	16	18	10	4	75	87	82
Arm. 1524	12	19	25	17	11	4	5	5	2	79	15	6	0	0	93	80	77
Arm. T909	20	12	18	22	8	14	4	2	2	83	9	5	2	1	93	86	82
PPV-13	13	16	27	19	13	2	3	6	1	82	12	4	1	1	93	83	79
PPV-21	23	7	19	10	11	11	5	5	2	79	15	6	0	0	93	80	77
Bruin-46	11	14	20	21	18	8	2	5	1	43	13	24	16	4	69	70	64
Bronco-90	9	16	14	13	17	7	7	11	6	43	30	22	4	1	78	80	74

chromosmes, or both, were lagging. Results show that most of the open bivalents at MI of hexaploid triticals resulted from desynapsis or terminalization of chiasmata which occurred after diakinesis (Fig. 1C, D). It is expected that chromosome association at diakinesis would be better than at MI in hexaploid triticales. At AI, univalents were distributed to the poles at random. They may pass to either pole without dividing at first division of meiosis and may subsequently divide normally at second division. Usually the lagging chromosome were not included in the nuclei after meiosis, but appeared as micronuclei in the quartet of spores (Fig. 1F, G). Occasionally the univalents divided at first division of meiosis and misdivided again at the second division. All nine strains of secondary triticales were meiotically abnormal, as indicated by tetrad analysis, with the percentage of normal tetrads ranging from 36 to 83 with a mean of 62. The number of micronuclei in various cells of the tetrads of immature pollen was obtained for each cultivar.

Five measures of reproductive stability were obtained for the nine triticale cultivars (Table 2). The earliest developmental stage when stability could be observed was during MI to AI where the number of lagging chromosomes was determened. The frequency of cells with no lagging chromosomes ranged from 6% for Arm-133 to 38% for 6TA-419. Even 6TA-204 and 6TA-419, which had the highest frequencies of cells with no lagging chromosomes, had substantial frequencies of cells with 1 to 5 lagging chromosomes. The nine cultivars had somewhat different distributions for the number of lagging chromosomes but differences appeared to be minor. The frequency of cells with 6, 7, or 8 lagging chromosomes was low for all cultivars, with the possible exception of Bronco-90.

At the tetrad stage of meiosis the frequency of tetrads with no cells having micronuclei was generally much higher than the number of cells without lagging chromosomes. For this measure the lowest frequency of normal tetrads was 36% (6TA-204) and the highest was 83% (Arm-T909). The classification of tetrads with micronuclei in 1, 2, 3, or 4 cells of the tetrad (Table 2) shows, that for all cultivars, micronuclei in 1 or 2 cells account for most of the meiotic abnormalities. Possible exceptions to this result were 6TA-204 and Bruin-46 which had 23 and 20% of the tetrads with micronuclei in 3 and 4 cells.

Mature pollen provided the final three measures of stability. Pollen without micronuclei ranged from 66% (6TA-204) to 93% (four cultivars). These results indicated greater stability than the previous estimate (percentage of normal tetrads). Stainability with I-KI and the frequency of pollen grains with three normal-appearing nuclei showed a higher degree of normality than

any of the other measures of viable pollen and 6TA-204 and 6TA-419 showed the highest frequencies for both measures. Percentage of mature, fertile pollen was based on the staining of pollen nuclei. The total frequencies of normal PMC's, good tetrad cells and completely developed pollen were listed in Table 2.

Correlations among the meiotic characteristics are listed in Table 3. These correlations were computed using the means (Table 2) for each meiotic characteristic for the nine genotypes. The correlations between meioses with no lagging chromosomes and good tetrad cells or with normal pollen (without micronuclei) were not significant. The correlation between tetrads and pollen with no micronuclei was very high (0.98**). Comparisons among lagging chromosomes, tetrad cells and mature pollen grains showed little or not relationship. It was found in meiotic division that lagging chromosomes included both uni- and bivalent chromosomes; thus all lagging chromosomes cannot be considered as abnormal. It was presumed the different time sequence of chromosomal replication occurred in the wheat-rye hybrid cells. Asa result there was a correlation between pollen stainability and tri-nucleate pollen in all materials but there was no correlation between micronuclei and pollen stainability. Normal meiotic chromsome separation was positively correlated to the production of functional pollen but not correlated with the number of micronuclei in pollen; this may be related to the different time sequence of chromosome replication in wheat-rye genome.

Table 3. *Correlation matrix of five meiotic characteristics of 9 42-chromosome triticales*

Character	Tetrads with 0 micronuclei	Pollen with 0 micronuclei	Pollen stained	Pollen with 3 nuclei
MI-AI with 0 lagging chromosomes	−0.04	−0.07	0.63	0.68*
Tetrads with 0 micronuclei		0.98**	−0.06	0.10
Pollen with 0 micronuclei			−0.16	−0.01
Pollen stained				0.69*

* $0.01<P<0.05$; ** $P<0.01$.

Meiotic Study of Intertriticale Hybrids

Eight combinations of intertriticale hybrids were examined for this study. In intercrosses of hexaploid triticales, associations of three, four, five and six chromosomes were quite frequent; in addition, a number of univalents and varying numbers of heteromorphic or univalent chromosomes were also found

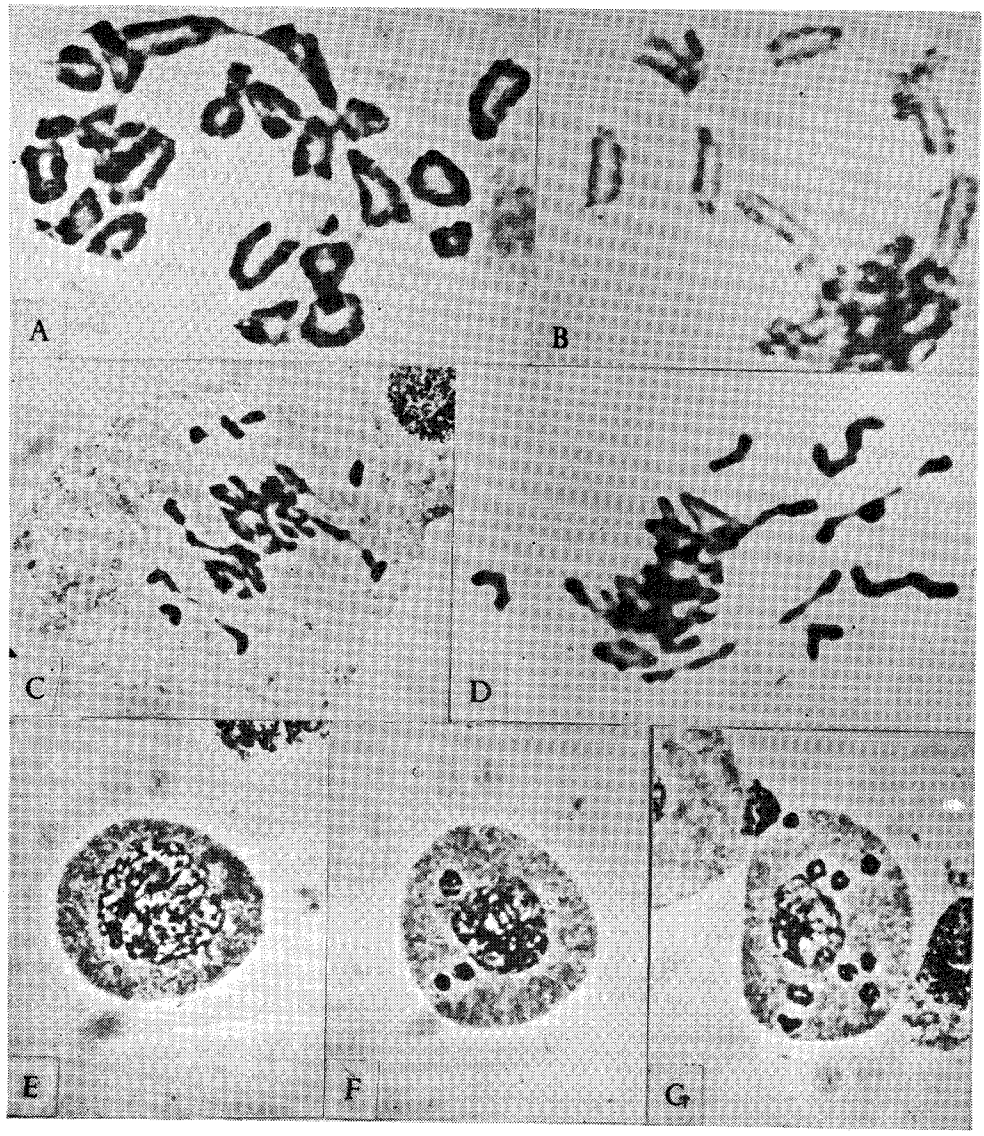

Fig. 1. Meiosis of 42-chromosome triticale.

A. 21 pairs of chromosomes in diakinesis stage (with association).
B. Early-late chromosome associations in diakinesis (different time sequences of replication).
C. Univalents, prematurely disjoined bivalent in MI.
D. Univalents, open bivalents in MI.
E. Normal pollen.
F. With three micronuclei in immature pollen.
G. With nine micronuclei in immature pollen.

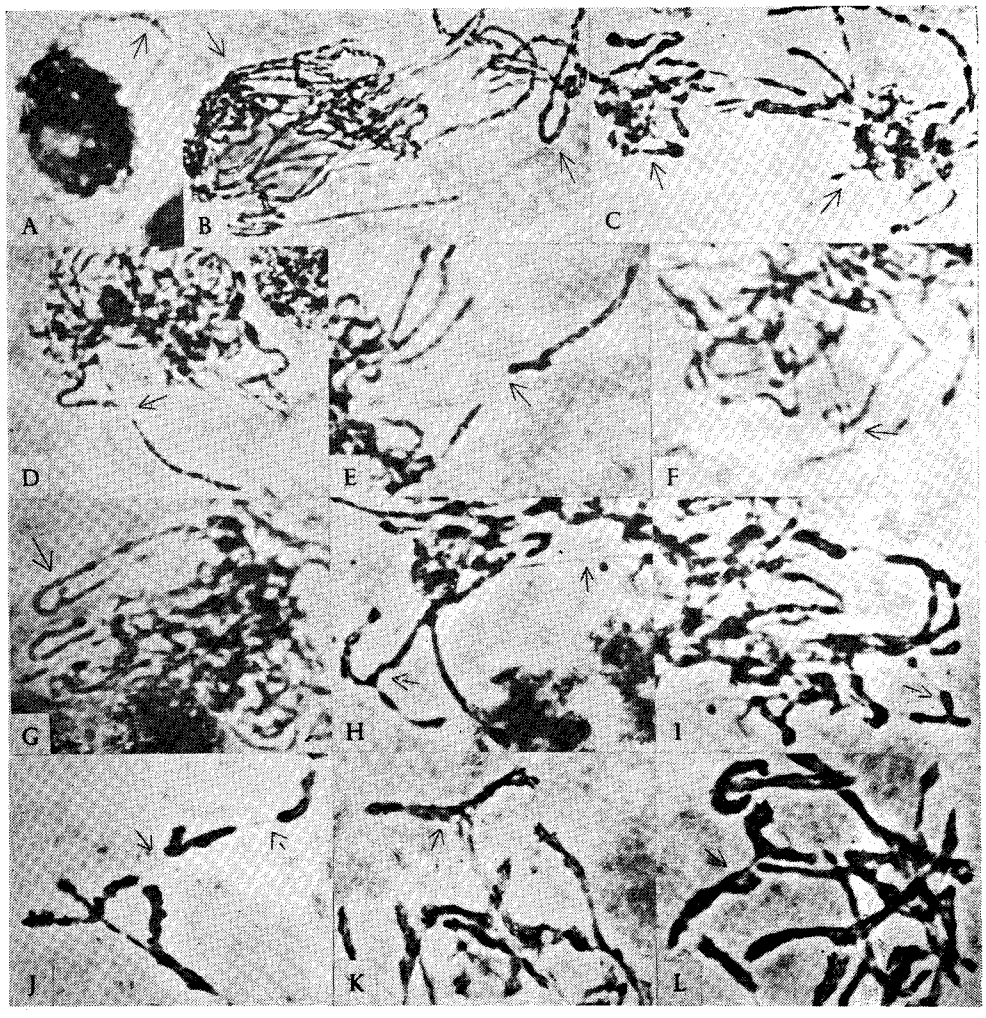

Fig. 2. Irregular chromosomes in meiotic division of intertriticals hybrids.
A. Chromosome in pachytene separated from zygotene.
B, C. Different time sequences of meiotic division in two separate clusters of chromosomes.
D, E. Lack of pairing in some portion of pachytene chromosome.
F. Desynapsis region in pachytene chromosome.
G. Unpaired loop region.
H. I. Trivalent, and univalent.
J. Multiple chromosomes associations.
K, L. Multivalent structure and interchange.

in some cells. Many univalents divided precocociously at MI and TI, and this may partially explain the high frequency of laggards noted at second telophase. One or more micronuclei were found in immature pollen in most cases, presumably comprised of the lagging univalents or bivalents of meiosis. In intertriticale hybrids, the complex configurations at meiosis were observed (Fig. 2) and chromosomal irregularity was indicated by absence of pairing or partial pairing in pachytene stage (Fig. 2D, E), desynapsis in a portion of the chromosome region (Fig. 2F), deletion, duplication or inversion loops (Fig. 2G), univalents and multivalents (Fig. 2H, I), chromosomal association (Fig. 2J), heteromorphic bivalents, complex multivalents, and interchanges (Fig. 2K, L).

The presence of complex multivalents and heteromorphic bivalents in hybrids of intercrosses would also be indication of considerable chromosomal rearrangement in the form of translocations in one or both of the parental groups. Some bivalents remained without disjunction even at the advanced stage of anaphase. This fact, together with the observance of a number of lagging chromosomes, suggests the lack of synchronization between the chromosomes in their meiotic organization. Second anaphase was also irregular with lagging chromosomes and chromatids. The telophase II and tetrad cells had a large number of micronuclei.

Discussion

The complex configuration at meiosis in secondary triticales could be due to several reasons. One possibility is that genetic system limiting pairing to homologous chromosomes breaks down in the hybrids, thus allowing both homoeologous and homologous chromosomes to associate at pachytene; another possibility is that the temperature-sensitive period is different between wheat and rye genomes. The temperature-sensitive stage occurs before DNA synthesis preceding meiosis in pollen mother cell nuclei. This implies that wheat and rye chromosomes differ in the initiation of meiosis. In the present investigation among secondary triticale lines it is also possible that they originate as a result of misdivisions. In intertriticale hybrids the presence of complex multivalents and heteromorphic bivalents may result in differences between lines in viability of gametes.

There are three types of bivalent chromosomes occurring in intertriticale hybrids, namely rod-, cross- and ring-bivalents which correspond to the three types studied by Schulz-Schaeffer (1971) in a *Triticum* × *Agropyron* hybrid. Side by side associations arise from complete asynapsis. Bivalent chromosomes were chromosomes were associated together by end chiasmata. Rod bivalents

consequently must be the result of incomplete pairing at synapsis of meiosis. All meiotic observations were in good agreement with the present investigation.

Another explanation for the extremely high frequency of aneuploidy in progeny populations of a particular strain is the extreme irregularity in meiotic chromosome behavior, resulting in a high percentage of aneuploid gametes and zygotes and environmental effect on meiosis. This was discussed by Tsuchiya and Larter (1969) and they suggested that chromosome studies should be included in the breeding procedure in triticale and, most likely all other synthetic amphiploids which are cytologically unstable.

Abnormal meiosis had an adverse influence on pollen grain viability and on fertility of plants. Seed setting in spikes depends on the strains and the individual plants. Fertility of florets in the spike also depends on vigor of the plant. Usally the main spikes show higher fertility than tillers. On the other hand, middle spikelets in the spike exhibit more satisfactory seed setting than the upper and lower ones and kernel development is significantly different between superior and inferior floret sites within a spikelet.

It is evident that natural selection has led to an increase in preferential pairing by selection toward increased differentiation among partly homologous chromosomes and eliminate the frequency of aneuploids under field conditions as a result of their low germination percentage or their poor emergence. Apparently, however, long continued selection is necessary to convert 'raw' amphidiploids into smooth functioning type such as *Triticum* species.

Some of these different genotypes may be adapted to new habitats, and have the potential to overcome an initial disadvantage in sterility by means of progressive diploidization. So it seems to be feasible to establish the triticale strains with highly stable meiosis by systematic selection accompanied by extensive and intensive cytological studies.

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次級型42染色體黑小麥減數分裂之探討

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黑小麥爲小麥與黑麥之雜種，是唯一人爲首育之穀類作物。利用細胞遺傳學之原理及方法，探討六元體黑小麥細胞減數分裂染色體行爲，以提供黑小麥育種之參考。從九個次級型黑小麥品系及其四個種間雜種，調查減數分裂單價體數，遲滯染色體數，四分子細胞額餘微核頻度花粉染色率及成熟有效花粉率（具三核）等性狀，發現有極大變異之不規則減數分裂行爲，其中遲滯染色體在親本中有呈 0～8 之差異，其減數分裂不規則之變異，顯係在染色體配對分離時，由於細胞中小麥及黑麥染色體組不同發生時序所致。黑小麥成熟有效花粉率與染色體減數分裂正常細胞頻度及花粉染色率有類著的正相關；但與具有額餘微核之四分子細胞無關，此證影響黑小麥結實率，減數分裂異常行爲是其中原因之一。