Cytological study on hypo-pentaploid *Triticale* with four B chromosomes of rye

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ABSTRACT

Hypo-pentaploid *Triticale* with four B chromosomes ($2n=33+4B$'s) derived from (*Triticum durum* $\times$ *Secale cereale* with $2B$'s) $\times$ *T. durum* was cytologically studied, particularly on the normal chromosome component and on the behaviour of B chromosomes.

As a result of Giemsa staining for meiotic metaphase in pollen mother cells it was made clear that the normal chromosome component in the present *Triticale* consisted of 27 chromosomes of *T. durum* and 6 ones of *S. cereale*. The present *Triticale* showed a highly disturbed meiotic pattern. At MI the modal configuration of chromosomes was found in $12_{II}+9_{I}+2B_{II}$. At AI the unequal distribution in number and the many lagging chromosomes were observed, but the micronuclei at tetrad were a small number.

The number of B chromosomes in the present *Triticale*, BF_1 plant, was twice as many as F_1 parent. This indicates that in *Triticale* the nondisjunction of B chromosomes also takes place as well as in the original rye plant. The degree of multivalent formation between the B chromosomes was much lower in the present *Triticale* than in the original rye plants with $4B$'s. This indicates that the meiotic pairing of B chromosomes is disturbed by the change of genetic environment.

1. INTRODUCTION

So far the cytological behaviour and genetical effects of B chromosomes in rye have been extensively studied in the diploid level by many investigators (Kishikawa 1965; Müntzing 1974), and also in the autopolyploid environment by Sarvella (1959), Müntzing (1963) and Kishikawa (1966). When considering a new reaction of B chromosomes, it seems desirable to rescrutinize the nature and function of B chromosomes in rye by transferring these chromosomes to an entirely new genetic environment. In this way, as described by Lindström (1965) in a posthumous paper formulated by Müntzing, it was possible to transfer B chromosomes of an allogamous species, *Secale cereale* L., to an autogamous species, *Triticum aestivum* L. The B chromosomes in allohexaploid wheat, the so-called Lindström strain, have been studied by Müntzing *et al.* (1969) and Müntzing (1970, 1973).

It would be interesting to transfer the B chromosomes in rye to an allotetraploid species of wheat, and to be compared with the data obtained on the

B chromosomes at the autopolyploid and allohexaploid level mentioned above. Therefore, crosses between allotetraploid wheat and rye with B chromosomes, and Lindström strain, have been carried out. In a series of studies, the present paper deals with the cytological studies on the hypo-pentaploid *Triticale* with four B chromosomes, which found in the backcrossed hybrids between tetraploid wheat and rye with B chromosomes, particularly on the normal chromosome component and the cytological behaviour of B chromosomes.

2. MATERIALS AND METHODS

The tetraploid wheat parent used in the crossing was *Triticum durum* Desf. var. *reichenbachii* Körn. ($2n=28$, AABB) and the rye parent a Japanese strain (JNK), *Secale cereale* L. (RR), with two standard B chromosomes ($2n=14+2$ B's). The resulting F_1 plants (ABR) with two standard B chromosomes ($2n=21+2$ B's) were backcrossed with wheat parent mentioned above. This BF_1 plant was used in the present investigation.

For observations of somatic chromosomes the excised root tips were pre-treated for about twenty hours in ice-water (ca. 0°C) and then fixed in alcohol-acetic acid (3:1). For studies of meiosis the young spikes were fixed in alcohol-chloroform-acetic acid (6:3:2). The cytological preparation for both root tip cells and pollen mother cells were made with a staining and squash method employing alcoholic hydrochloric acid-carmines described by Snow (1963). Identification of rye chromosomes in the BF_1 plant was made with Giemsa staining technique in meiotic chromosomes described by Singh and Lelley (1975).

3. RESULTS AND DISCUSSION

At mitotic and meiotic metaphase B chromosome can easily be distinguished by its smaller size and subterminal centromere position from normal chromosomes. All of B chromosomes occurred in the present materials were a standard type. The somatic chromosomes of F_1 and BF_1 plants, as shown in Figs. 1 and 2, were $2n=21+2$ B's and $2n=33+4$ B's, respectively. The number of normal chromosomes in BF_1 plant was less by 2 than eu-pentaploid number, 35. This indicates that the present BF_1 plant is a hypo-pentaploid *Triticale* having four B chromosomes.

In order to elucidate the normal chromosome component in the present *Triticale*, Giemsa staining technique was employed for meiotic metaphase chromosomes. Consequently, as shown in Fig. 3, 6 of univalents showed distinct band at one end or both ends (cf. Lelley 1975). They are clearly the chromosomes of rye. This indicates that 33 of chromosomes in the present *Triticale* consist of 6 chromosomes of rye and 27 ones of wheat. Because the chromosome complement ($n=14$) in the gametes of tetraploid wheat used as the recurrent parent is entirely normal, the numerically aberrant chromo-

Figs. 1 and 2. Somatic chromosomes in root tip cells of wheat-rye hybrids. (Arrows indicate B chromosomes.)

Fig. 1. F_1 plant: $2n=21+2$ B's. Fig. 2. BF_1 plant: $2n=33+4$ B's.

Fig. 3. 6 univalents of rye (arrows) recognizable by terminal Giemsa bands at meiotic metaphase I in BF_1 plant.

somes ($n=19$) resulted from the irregular gametogenesis in F_1 plant (Kishikawa and Nagai, unpublished; cf. Nakajima 1951, 1952) may be contributed to the occurrence of the present aneuploid.

On the other hand, the present BF_1 plant had twice as many B chromosomes as F_1 parent. This is due to the non-disjunction of B chromosomes occurred in

Table 1. *Chromosome pairing at metaphase I in hypo-pentaploid Triticale with four B chromosomes*

Configurations		No. of cells observed
Normal chromosomes	B chromosomes	
13 _{II} + 7 _I	1 _{IV}	1
" "	2 _{II}	1
12 _{II} + 9 _I	1 _{IV}	20
" "	1 _{III} +1 _I	1
" "	2 _{II}	50
" "	1 _{II} +2 _I	10
" "	4 _I	1
11 _{II} +11 _I	1 _{IV}	4
" "	2 _{II}	8
" "	1 _{II} +2 _I	4
10 _{II} +13 _I	2 _{II}	2
" "	4 _I	1
9 _{II} +15 _I	2 _{II}	2
Total		105

Figs. 4 and 5. Chromosome configurations at meiotic metaphase I in hypo-pentaploid *Triticale* with four B chromosomes. (Arrows indicate B chromosomes.)Fig. 4. 12_{II}+9_I+1 B_{IV}. Fig. 5. 10_{II}+13_I+2 B_{II}.

the ovules of F₁ parent. This indicates that in *Triticale* the non-disjunction of B chromosomes also takes place in the first pollen mitosis and the corresponding stage in the ovules, just as in the original rye plant (Müntzing 1946; Kishikawa 1965) and Lindström strain (Müntzing 1970).

The present *Triticale* showed a highly disturbed meiotic pattern.

Data on the chromosome pairing at meiotic metaphase I are summarized in Table 1, and the configurations are shown in Figs. 4 and 5. The modal con-

Table 2. Frequency of *B* chromosome pairing at MI in the different plants with four *B* chromosomes

Configurations	Plants with 4 B's		
	5x-2 <i>Triticale</i>	2x Rye*	3x Rye*
1 _{IV}	23.8%	74.0%	54.5%
1 _{III} + 1 _I	1.0	3.0	2.5
2 _{II}	60.0	21.4	40.0
1 _{II} + 2 _I	13.3	1.6	3.0
4 _I	1.9	0.0	0.0
No. of cells	105	800	200

Note: * These data were quoted from the data of Kishikawa (1966).

Table 3. Frequency of *B* bivalents at MI in *F*₁ plants (*T. durum* × *S. cereale* with 2*B*'s) and the original rye plants with two *B* chromosomes

Configurations	<i>F</i> ₁	Rye
1 _{II}	28.5%	92.4%
2 _I	71.5	7.6
No. of cells	260	630

figuration of chromosomes was found in $12_{II} + 9_I + 2B_{II}$. As for the pairing of normal chromosomes, the number of bivalent per cell ranged from 9 to 13 with an average of 11.8, and the univalent from 7 to 15 with an average of 9.4. All bivalents are considered to be based on the allosyndesis among chromosomes of wheat parent, because as a result of Giemsa staining the chromosomes of rye behave always as the univalents (Fig. 3). The maximum class of configuration, $13_{II} + 7_I$, is composed of 13 bivalents and 1 univalent of wheat chromosomes, and 6 univalents of rye. In other words, the present aneuploidy is due to the loss of one chromosome of wheat and rye, respectively.

As for the pairing of *B* chromosomes, the configurations and their frequencies were summarized in Table 2 by extracting the data from Table 1, in comparison with the data from the original rye plants (JNK strain), both diploid and triploid with four *B* chromosomes, reported previously (see Kishikawa 1966, p. 430). In the present *Triticale* *B* chromosomes paired fairly well with one another, and the same configurations as in the original rye plants were observed. The quadrivalent also was characterized by radial association. There is, however, a great difference between the degree of pairing of the *B* chromosomes in the original rye plants and in the present *Triticale*. That is, the frequency of quadrivalent and trivalent was much lower in the present

Table 4. *Chromosome separation at anaphase I in hypo-pentaploid Triticale with four B chromosomes*

Distributions		No. of cells observed
21	16	1
20	17	2
19	16	1
Laggards:		
20 1	16	1
17 4	16	1
16 5	16	1
17 6	14	2
16 6	15	2
18 7	12	1
16 7	14	6
15 7	15	2
15 8	14	17
15 9	13	8
14 9	14	27
14 10	13	12
14 11	12	2
13 11	13	8
15 12	10	1
13 12	12	4
11 15	11	1
Total		100

Figs. 6 and 7. Chromosome distributions with laggards at meiotic anaphase I in hypo-pentaploid *Triticale* with four B chromosomes.

Fig. 6. 17-6-14. Fig. 7. 14-10-13.

Triticale than in the original diploid and even the triploid plant, while the one of bivalent and univalent higher. This indicates that the multivalent formation of *B* chromosomes in the present *Triticale* is far inferior to that in the original rye plants. In this connection, Kishikawa and Nagai (unpublished) have found that in F_1 plant with two *B* chromosomes, a parent of the present *Triticale*, the frequency of *B* bivalents was strikingly lower than in the original rye plants with two *B* chromosomes, as is evident from Table 3. One possible cause of the low frequency of meiotic pairing between the *B* chromosomes may be a disturbance of the pairing of *B* chromosomes with the irregular division at meiosis in polyhaploid (F_1) and aneuploid (BF_1). Müntzing *et al.* (1969) also have reported that the degree of meiotic pairing of the *B* chromosomes was much lower in Lindström strain in which the *B* chromosomes of rye occur together with the 42 wheat chromosomes than in the original rye variety, "Transbaikal". This fact is essentially identical to the present result, although both materials differ much in the genotypical constitution from each other. The most plausible cause of the reduced meiotic pairing of the *B* chromosomes, as Müntzing pointed out, seems to be a disturbing effect of the wheat chromosomes or its cytoplasm. And also, it has been reported that the pairing of *B* chromosomes in rye was much poorer in the autotetraploid (Sarvella 1959) and in the autotriploid (Kishikawa 1966) than in the original diploid.

Judging from the facts described above, it seems most reasonable to conclude that the meiotic pairing of *B* chromosomes is disturbed by the change of genetic environment, such as from diploid to autopolyploid in rye and from rye to wheat.

The chromosome segregation at meiotic anaphase I in the present *Triticale* was strikingly irregular. The data are summarized in Table 4, and the distribution of chromosomes is shown in Figs. 6 and 7. In all of AI cells the unequal distribution in number and many lagging chromosomes were observed. The number of lagging chromosomes ranged from 1 to 15 with an average of 8.5. In this stage it was difficult to distinguish *B* chromosomes from normal ones. Normal chromosomes, however, may be responsible for most of lagging chromosomes as a result of the predominant occurrence of univalent at metaphase I. The lagging chromosomes may lead to the occurrence of micronuclei

Table 5. *Number of micronuclei at tetrad stage in hypo-pentaploid Triticale with four B chromosomes*

	Number of micronuclei per tetrad								Total
	0	1	2	3	4	5	6	7	
No. of tetrads	36	48	114	30	40	23	5	4	300
%	12.0	16.0	38.0	10.0	13.3	7.7	1.7	1.3	100

at tetrad stage. Actually, in most of cells at this stage the micronuclei were observed. The number of micronuclei is given in Table 5 as the sum of micronuclei included in four daughter cells. Their number ranged from 1 to 7 with an average of 2.3. In spite of a large number of occurrence of the lagging chromosomes at anaphase I, the number of micronuclei at tetrad stage was much fewer than expected. This indicates that most of lagging chromosomes may reach finally the daughter nuclei at telophase I and II.

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