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Cytological Investigation of *Triticale*

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Summary. A cytological investigation of 15 different 56-chromosome *Triticale* and 16 *Triticale* with 42 chromosomes was carried out. 4 were primary *Triticale* and 12 were secondary *Triticale*. Chromosome pairing was not disturbed; 21 and 28 bivalents were found in the hexaploids and octoploids, respectively. Meiotic irregularities were established, however, in all the *Triticale* studied; in octoploids the frequency of the irregularities was 22–88% and in hexaploids it was 12–87%.

In metaphase and anaphase asynchronous separation of chromosomes was noted. Incompatibility between wheat and rye genomes and the inactivation of single loci of rye chromosomes are suggested as the main causes of the irregularities in meiosis.

Mitotic disturbances were found in all the amphidiploids. The frequency of anomalies in mitosis was considerably lower than in meiosis: in octoploids they made up 5%–11% and in hexaploids 6.2%–15.2%. In all the amphidiploids studied chimera plants were found containing pollen mother cells with different chromosome numbers. The chromosome number in the aneuploid cells varied from 8–48 in hexaploids and from 8–62 in octoploids. Octoploid *Triticale* had 29.4%–72.9% aneuploid pollen mother cells, while hexaploid *Triticale* had 5.2% –55.7%.

Wheat-rye amphidiploids (Triticale) have been known for about 80 years. The first Triticale with 56 chromosomes was obtained by Rimpau in 1891. A large number of wheat-rye amphidiploids started to appear, particularly in the 1930's and subsequent years (Meister, 1930; Müntzing, 1936, 1939, 1956, 1964; Pissarev, 1947, 1963, 1964; O'Mara, 1940, 1951; Riley and Chapman, 1957). During the past 10 to 15 years there has been extensive research on Triticale with 42 chromosomes. The possibility of producing such Triticale was shown as early as 1937 (Derjavin, 1938; Nakajima, 1953; Sanchez-Monge, 1956; Shulindin and Naumova, 1965, 1966; Pissarev and Jilkina, 1965, 1967). At present, Triticale have been obtained in England, Bulgaria, Hungary, German Democratic Republic, Canada, France, German Federal Republic, U.S.A., Japan and other countries. In the U.S.S.R. 98 plant breeding stations and scientific institutions are studying different Triticale.

Triticale possess a number of advantages of practical interest to the breeder: increased protein content; a high content of grain crude gluten; a larger spike with a high number of spikelets; resistance to certain fungal diseases. The use of Triticale as commercial varieties is hampered, however, by poor seed setting. Attempts to improve the fertility of octoploid Triticale have failed and these Triticale are of interest as breeding material only. Hexaploid Triticale proved to be more promising. Some of them have good seed setting and when sown on poor sandy soil have a number of advantages compared with other cereals (Makhalin, 1965; Kiss, 1966; MacDonald, 1968).

It has been indicated that the cause of the partial sterility of Triticale is the appearance of aneuploids (Krolow, 1962, 1963, 1966; Kappus, 1964). Since the appearance of aneuploids is necessarily related to meiotic irregularities, cytological studies of Triticale are of definite interest.

Up to now, cytological analysis has included only the determination of the percentage of irregular tetrads having micronuclei and polyads, and, at best, the number of univalents in M_I of meiosis has been estimated (Levitsky, Benetskaya, 1930; Avdulov, 1937; Berg and Oehler, 1938; Vettel, 1959, 1960a, 1960b). It has been suggested that two different chromosome complements derived from different parental species or varieties cannot function as a single unit in Triticale (Florell, 1936; Riley and Chapman, 1957, 1958; Riley and Law, 1965). However, there is a divergence of opinion as to the causes of meiotic irregularities in Triticale. Müntzing (1955, 1956) considers that the higher the chromosome number in Triticale, the commoner the disturbances in meiosis. He has developed decaploid Triticale with 70 chromosomes; there were more irregularities in the decaploids than in octoploids ($2n = 56$). Müntzing attributed this to the doubling of the rye genome and concluded that the ratio between the numbers of different genomes affects the course of meiosis. Inbreeding depression of the rye genome is Müntzing's (1958) another explanation for this observation. Lacadena (1965) shares this viewpoint and attributes the low fertility of Triticale to the incompatible genes of rye. Krolow (1963) holds that a mechanism preventing the normal pairing of chromosomes underlies meiotic irregularities in Triticale. Since meiotic irregularities cannot be explained by the lack of chromosome homology, Krolow suggests that certain genes of wheat influence the rate of chiasmata

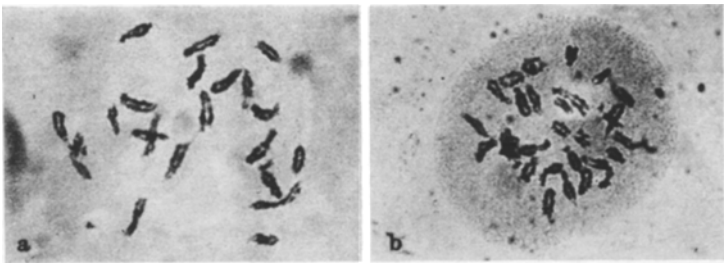

Fig. 1. Chromosome pairing (diakinesis) in hexaploid and octoploid Triticale: a) 2111; b) 2811

formation in rye chromosomes or prevent their pairing. Thus the causes of meiotic irregularities in Triticale remain unclear.

The present paper reviews the cytological studies on wheat-rye amphidiploids carried out in the Laboratory of Cytogenetics of the Institute of Cytology and Genetics of the Siberian Department of USSR Academy of Sciences. Problems concerning aneuploidy have been omitted so far, as they have been analyzed at length by Krolow (1962, 1963, 1965, 1966) as well as in the articles we have published elsewhere (Shkutina, Golubovskaja and Khvostova, 1967; Shkutina, 1969).

Materials and Methods

15 different 56-chromosome Triticale and 16 42-chromosome Triticale, of which 4 were primary and 12 secondary, were used in the study. 56-chromosome amphidiploids H34AD, 31 AD72, 25AD20, H120AD, 10H120AD, H121AD, H129AD, HAD330 were obtained by V. E. Pissarev and 5202, 5203, 5208 were obtained by M. A. Makhalin, from *Triticum aestivum* × *Secale cereale* crosses. Derjavin Triticale was produced by crossing *Triticum aestivum* × *Secale montanum*. Amphidiploid 529AD117 was produced by Müntzing. 42-chromosome primary amphidiploids from Hungary, Canada, Spain and USSR (Derjavin Triticale) were obtained from crosses of tetraploid wheats with rye. 42-chromosome secondary amphidiploids 5544, 5573, 5312, 4238, 4217, 4214, 4209, 4319, 4149, 4190, 4223, 5532 were derived by Makhalin from crosses between octoploid ($2n = 56$) and hexaploid ($2n = 42$) Triticale.

Newcomer's fixative was used for fixation of meiosis. Cytological studies were carried out on acetocarmine squash preparations. Mitosis was studied on temporary root tip squashes. The tips were treated in Carnoy's fixative (3:1) and stained by Feulgen's method. The number and the size of the nucleoli were determined in the prophase of meiosis. The material was fixed in a mixture of alcohol and glacial acetic acid (3:1). Permanent preparations were made by staining transversal microscopic sections of anthers 10 μ thick with methyl green pyronin. The size of the nucleoli were determined on temporary preparations stained with acetocarmine. Using a drawing apparatus, the outlines of 50 nucleoli of each amphidiploid were sketched. The drawings were cut out and weighed. The average weight of each nucleolus was determined in conditional units. Nucleolus formation was studied on meristematic cells of root tips. Fixation was by the method of Rattenbury (1952). Acetocarmine stain was used. Squash preparations were studied. The size of the nucleoli was determined by weighing the drawings of the nucleoli, and the average weight of a nucleolus per telophase was established. Since a high number of aneuploids are observed within Triticale populations, after germination one root from each seed was fixed for determining chromosome number. In studies of nucleoli, the roots were selected only from the seedlings that proved to be euploids. To determine the number of nucleolar organizers in wheat-rye amphidiploids the actual number of nucleoli per interphase nucleus was compared with the theoretically expected one.

Results

Octoploid Triticale have the complete chromosome set of common wheat — 42 and the complete chromosome set of rye — 14. Hexaploid Triticale also include two complete wheat genomes — 28 and a complete chromosome set of rye — 14. Thus in the cells of Triticale each chromosome has a corresponding homologue and pairing in the prophase of meiosis should be normal. To check this suggestion, it was established whether bivalents are present in diakinesis of octoploids and hexaploids. Pairing was studied in 6 octoploids (H34AD, 31AD72, H120AD, H121AD, 25AD20, 5202) and 6 hexaploids (5544, 5573, 4238, Hungarian, Canadian, AD Derjavin). 10 plants of each amphidiploid (15—20 cells per slide) were studied. Only in one plant within Triticale 25AD20 were multivalents and univalents found in M_1. In all the other amphidiploids, only bivalents were found at diakinesis: 28 in octoploids and 21 in hexaploids (Fig. 1). Occasionally 1—3 rod bivalents were observed. This implies that chromosome pairing in Triticale proceeds normally. The formation of multivalents is attributable to chromosome substitution: although the plant possesses 56 chromosomes, it has 3 homologous chromosomes instead of one chromosome pair and there is only one chromosome in place of the other chromosome pair.

In spite of normal chromosome pairing, a high frequency of meiotic irregularities was typical of all the Triticale (Table 1). The disturbances were observed in all stages of meiosis: in M_1, chromosomes outside the equatorial plate were seen; in A_1 and A_{II}, lagging chromosomes and sometimes bridges were observed; there were many micronuclei in telophases and polyads were noted as well (Fig. 2). Table 1 shows that the frequency of meiotic irregularities ranges from 22%—88% in octoploids and from 12%—87% in hexaploids. The frequency of meiotic irregularities in secondary 42-chromosome Triticale did not differ from that in primary ones. In both primary and secondary Triticale, forms with high and low degrees of meiotic irregularities were established. It should be noted that the amphidiploids differed not only in the frequency, but also in the type of meiotic irregularity. In some Triticale, a substantial proportion of the irregularities consisted of disturbances in spindle formation so that a high number of polyads arose. Thus, in amphidiploid H34AD, of the total number of irregularities 21% consisted of polyads, and in amphidiploid 31AD72,

Table 1. *Frequency of meiotic irregularities in 56- and 42-chromosome Triticale*

Triticale	2n	Number of		Number of tetrads with micronuclei	Number of polyads		Frequency of irregularities %
		plants	tetrads		total	%	
AD from Canada	42	11	1000	615	1	0.2	61.5 ± 1.54
AD from Spain	42	17	1700	951	3	0.3	55.9 ± 1.20
AD from Hungary	42	26	2400	1021	49	4.8	42.5 ± 1.01
Derjavin AD	42	6	600	322	3	0.9	52.7 ± 2.03
5544	42	8	800	592	—	—	73.9 ± 1.39
5573	42	7	400	351	—	—	87.7 ± 1.64
5312	42	9	900	108	—	—	12.0 ± 0.09
4149	42	7	500	125	1	0.9	25.0 ± 2.50
4139	42	9	500	127	3	2.5	25.4 ± 1.95
4223	42	4	400	174	—	—	43.5 ± 2.48
4190	42	2	200	88	—	—	44.0 ± 3.61
4138	42	5	500	281	—	—	56.3 ± 2.52
4209	42	6	600	213	—	—	35.5 ± 1.95
4217	42	5	400	300	—	—	75.0 ± 1.92
4214	42	5	500	292	—	—	58.5 ± 2.18
5539	42	5	500	209	—	—	41.8 ± 2.86
31AD72	56	4	2092	458	196	30	31.2 ± 1.02
H34AD	56	6	2745	577	153	21	26.6 ± 0.84
H120AD	56	6	1591	615	19	3	39.8 ± 1.23
10H120AD	56	3	2514	974	39	3.8	40.2 ± 0.98
H121AD	56	3	1256	479	9	2.0	38.9 ± 1.37
H129AD	56	2	1384	668	13	1.9	49.2 ± 1.36
HAD330	56	6	600	323	15	4.4	56.3 ± 2.02
25AD20	56	11	1100	497	68	12.1	50.8 ± 1.50
529AD117	56	8	3510	447	37	4.7	22.3 ± 0.70
5202	56	9	900	516	13	2.5	58.8 ± 1.64
5203	56	5	500	249	3	1.2	50.4 ± 2.23
5208	56	4	400	343	9	2.6	88.0 ± 1.62
5211	56	10	1000	424	5	1.2	42.9 ± 1.56
AD Rimpau	56	3	300	207	4	1.8	73.7 ± 2.98

polyads made up 30% of the total number of irregularities. All the other amphidiploids had 0—12% of polyads. In hexaploid amphidiploids, disturbance of spindle formation was observed less often than in octoploids. Only Hungarian AD had polyads making up 4.8% of the total number of irregularities. No polyad formation was noted in most hexaploid Triticale.

As mentioned above, regular chromosome pairing was observed in all the Triticale at diakinesis. However, in M_1 a pattern was established that can be interpreted as either chromosome lagging or the presence of univalents. We suggest that chromosomes in M_1 lying outside the metaphase plate were not univalents but chromosomes of precociously separated bivalents. This may be confirmed by the observation that chromosomes lying outside the metaphase plate were mostly identical in size and shape. Moreover, they frequently lay symmetrically in relation to the metaphase plate (Fig. 2). Lagging chromosomes were commonly observed at the equator in A_1 (Fig. 2). The impression was formed that some of the chromosomes of these amphidiploids have a different rhythm of cell cycle. The comparison of irregularity frequencies at different stages of meiosis confirms this impression. Table 2 presents the data on irregularity frequencies at different meiotic stages in one octoploid and one hexaploid Triticale. The table shows that in both amphidiploids irregularity frequencies fell from metaphase to telophase in first as well as second divisions. In M_1 of Triticale H34AD, abnormalities were observed in 60.6% of the M_1 cells, whereas in dyads, only 20.4% of abnormal cells occurred. The same pattern was observed in the second division: in M_{II} 50.3% cells with abnormalities were noted, in

Table 2. *Frequency of irregularities at different meiotic stages in one octoploid and one hexaploid Triticale*

Meiotic stage	Number of studied		Number of abnormal cells	
	plants	cells	total	%
	Derjavin Triticale (2 n = 42)			
MI	7	601	341	56.8 ± 2.02
AI	7	300	178	59.3 ± 2.91
D	7	309	134	44.6 ± 2.83
MII	7	700	433	61.8 ± 1.83
AII	7	588	283	47.6 ± 2.06
T	7	511	226	45.2 ± 2.20
	Triticale H34AD (2 n = 56)			
MI	6	1984	1204	60.6 ± 1.20
AI	6	543	264	48.6 ± 2.16
D	6	1090	222	20.4 ± 1.27
MII	6	2255	1135	50.3 ± 1.05
AII	6	1329	582	43.8 ± 1.36
T	6	2745	730	26.6 ± 0.84

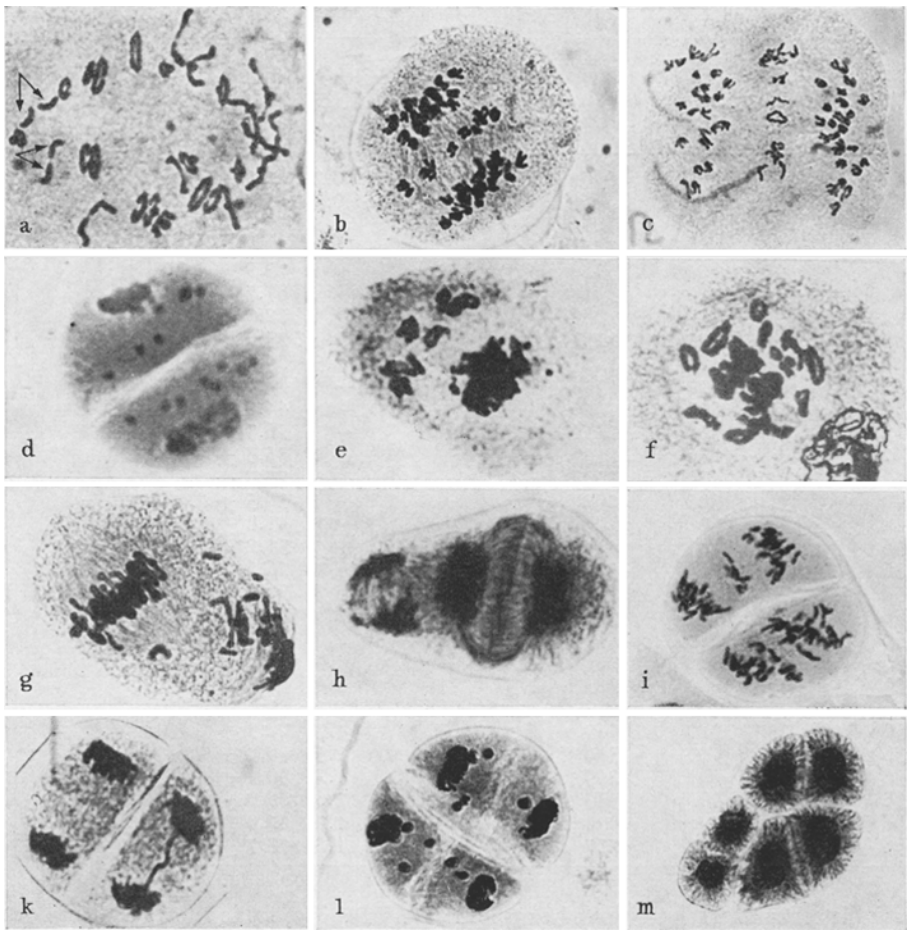

Fig. 2. Meiotic irregularities in Triticale: a) single chromosomes in M_I; b) chromosomes lagging at the equator in A_I; c) A_I, lagging chromosomes are seen, each of them is divided into two chromatids; d) 14 micronuclei in dyad; e) 7 bivalents in prophase lying separately from the other chromosomes; f) prophase with 2 nuclei having different degrees of coiling; g) two spindles in M_I; h) two spindles in A_I; i) chromosomes lagging in A_{II}; k) bridge in A_{II}; l) micronuclei in tetrad; m) polyad

tetrads the corresponding percentage was 26.6%. In Triticale Derjavin the same distribution of aberrant cells was established. It seems that not all the chromosomes lagging in A_1 form micronuclei. At telophase, they are partly included into the nucleus of the dyad; in M_{II} and A_{II} the laggards appear again, but at telophase they are also included partially into the nuclei of the tetrads. The number of chromosome laggards varied, but never exceeded 14. Fig. 2 reproduces 14 micronuclei that were probably formed from the 14 lagging chromosomes. It is suggested that rye chromosomes included into Triticale cells have a different cell cycle from that of wheat chromosomes. These differences are well displayed at the prophase stage, where a number of chromosomes differing in degree of coiling were noted. Fig. 2 depicts the prophase in which the majority of the chromosomes, evidently wheat chromosomes, are in the diakinesis stage, whereas a minority, probably rye chromosomes, are at the pachytene stage. It is of interest that the rye genome in Triticale can not only lag behind the wheat genome in the cell cycle, but lies apart as well, i.e. it forms a separate nucleus at prophase, organizes its own spindle in M_1 and separates independently in A_1 (Fig. 2). 7 bivalents lying separately from the main mass of the nucleus are seen in Fig. 2. All these events were observed in octoploids as well as hexaploids. As mentioned above, some amphidiploids differ from each other in the type of meiotic irregularity. In some Triticale, a considerable part of the disturbances consists of abnormal spindle formation leading to polyads. In these amphidiploids, rye chromosomes lying apart from wheat chromosomes are very rare; in the other amphidiploids, the main meiotic irregularity is chromosome lagging leading to the formation of micronuclei. In such Triticale, rye chromosomes lying apart from wheat chromosomes are more frequent, occurring in about 12% of the cells.

Chromosome number was estimated in different cells of an anther in metaphase I of meiosis. It was found to be unstable. It proved that for the most part the plants in populations of Triticale are chimeras. The percentage of aneuploid cells varied from 29.4% to 72.9% in octoploids and from 5.2%—55.7% in hexaploids; chromosome number in aneuploid cells ranged from 8—62 in octoploids and from 8—48 in hexaploids (Table 3). The chromosomes were randomly distributed in aneuploid cells; this is attributable to the irregular distribution of the chromosomes between the cells of polyads and to disturbed spindle organization.

In an attempt to understand the causes of the variation in the chromosome cycle in Triticale, nucleolus formation in Triticale cells was traced. Nucleolus formation gives an indication of the active or inactive states of the nucleolar organizer in the chromosome. Nucleolus formation was studied more thoroughly in Triticale H121AD and 31AD72. These

Table 3. *The number of aneuploid cells and variation of chromosome number in meiotic aneuploid cells of different Triticale*

Triticale	2n	Number of		Number of aneuploid cells		Variation of chromosome number in aneuploid cells
		plants	cells	total	%	
AD from Canada	42	17	87	33	37.9 ± 5.21	12–48
AD from Spain	42	3	19	9	47.1 ± 3.6	8–40
AD from Hungary	42	26	163	40	23.9 ± 3.37	16–43
5544	42	8	90	25	27.7 ± 4.71	34–44
5573	42	7	131	73	55.7 ± 4.35	20–46
5312	42	9	167	46	27.5 ± 3.34	8–44
5539	42	5	54	13	24.1 ± 5.81	38–40
4149	42	7	70	9	12.8 ± 3.98	24–44
4139	42	9	90	23	25.5 ± 4.59	36–44
4223	42	4	29	3	10.3 ± 5.65	38–40
4190	42	2	20	3	15.0 ± 2.48	38–40
4138	42	5	42	5	11.9 ± 4.99	36–40
4209	42	6	58	3	5.2 ± 2.91	36–38
4217	42	5	36	3	8.1 ± 4.58	38–40
4214	42	5	41	16	43.9 ± 2.46	38–44
H34AD	56	26	115	42	37.8 ± 4.51	8–58
31AD72	56	40	214	63	29.4 ± 3.11	22–62
H121AD	56	20	85	62	72.9 ± 4.81	14–62
HAD 330	56	8	43	24	55.8 ± 7.56	24–58
25AD20	56	44	209	93	44.4 ± 3.42	44–62
5205	56	12	189	112	59.2 ± 3.56	18–60
5203	56	5	76	42	55.2 ± 5.70	40–54
5211	56	10	109	67	61.4 ± 4.65	22–60
AD Rimpau	56	5	58	33	56.2 ± 5.61	42–54

differ from each other in the type of meiotic irregularity. The parents of these Triticale, wheat var. Lutescens 0329 and rye Jitkinskaya, were studied. Table 4 shows that wheat has one nucleolus in the nucleus with the average weight of 9.5 ± 0.06 conditional units according to the drawing of the nucleolus; rye also possesses one nucleolus, although a smaller one (4.7 ± 0.01). Triticale 31AD72, showing frequent disturbance of spindle formation in meiotic division, has one nucleolus equal in size to the sum of the sizes of wheat and rye nucleoli (13.4 ± 0.91). Triticale H121AD, which has the highest number of lagging chromosomes in meiosis, has generally one nucleolus not differing in size from the wheat nucleolus (10.2 ± 0.11). However, occasionally the nuclei of this amphidiploid were observed to contain 2 nucleoli similar in size to wheat and rye nucleoli (10.0 ± 0.16 and 4.8 ± 0.54). The results of studies of diakinesis show that in rye one pair of chromosomes participates in nucleolus formation, while in wheat 3 pairs of chromosomes do so; in Triticale 31AD72, which has one common nucleolus, 4 pairs of chromosomes are involved in nucleolus formation, whereas in Triticale H121AD, with 2 nucleoli, 3 pairs of chromosomes take part in the formation of the large (wheat) nucleolus and one pair participates in the formation of the smaller (rye) nucleolus. In some plants of this Triticale, at the prophase of meiosis one nucleolus is formed equal in size to that of wheat; 3 chromosome pairs, probably wheat chromosomes, are involved in its formation (Fig. 3).

The study of nucleous formation in mitosis showed that it proceeds in Triticale just as in other plants. The nucleolus disappears in prophase. Traces of nucleolar substance are occasionally noted in prometaphase, but none is distinguishable at metaphase. Pronucleolar substance appears in anaphase as droplets or layers between the chromosomes. Nucleolar substance is condensed into large drops during telophase. By the end of telophase, all the nucleolar substance is concentrated in the region of nucleolar organizers. Differences were established in the process of nucleolus formation in the Triticale studied. In Triticale 31AD72, which has one common nucleolus in meiosis formed by wheat and rye chromosomes, the nucleolar substance is accumulated by

Table 4. *The number and size of nucleoli and number of chromosome pairs, involved in nucleolar formation*

Name	Number of nucleoli	Size of nucleoli (conditional units)	number of chromosome pairs
wheat var. Lutescens 0329	1	9.5 ± 0.06	3
rye var. Jitkinskaya	1	4.7 ± 0.01	1
Triticale H120AD	1	10.2 ± 0.11	3
Triticale H120AD	2	10.0 ± 0.16 and 4.8 ± 0.54	3 1
Triticale 31AD72	1	13.4 ± 0.91	4

Table 5. *Quantity of nucleolar substance in one telophase (conditional units) in mitosis*

Triticale	Number of nucleoli in telophase	Number of telophases studied	Quantity of nucleolar substance	t_{dif}
31AD72	4	50	12.8±0.04	1.43
31AD72	3	50	12.7±0.07	
H121AD	4	50	12.1±0.05	
H121AD	3	50	10.5±0.03	26.7
H121AD	3+drops	50	10.2±0.03	2.26

all the chromosomes. At telophase, pronucleolar material is condensed in to large drops, and 4 nucleoli are usually formed (Fig. 3). Since this Triticale has 4 pairs of nucleolar organizers, the appearance of 4 nucleoli at telophase quite accords with what is expected. To explain the appearance of 3 nucleoli in telophase, nucleolar material in cells with 3 and 4 nucleoli was compared. The data in table 5 show that nucleolar material in telophase with 4 nucleoli is equal to 12.8 ± 0.04, and at telophase with 3 nucleoli it is 12.7 ± 0.07. There are no significant differences between these estimates. Thus the nucleoli in telophase have fused to produce 3 nucleoli, i.e. in these 3 nucleoli, pronucleolar material derived from all chromosomes has accumulated. The actual distribution of the number of nucleoli per interphase was compared with the expected distribution for 4 pairs of nucleolar organizers, in order to confirm the evidence for the involvement of 4 chromosome pairs in nucleolus formation in this Triticale (Table 6). It proved that the actual distribution corresponds entirely to the one expected on theoretical grounds. This indicates that 4 pairs of nucleolar organizers function in the cells of this Triticale.

In Triticale H121AD another pattern was found. Only 1 nucleolus was noted. Only 3 pairs of wheat chromosomes participated in the formation of this nucleolus, but in some cells a rye nucleolus was formed too; however the nucleolar organizer of rye func-

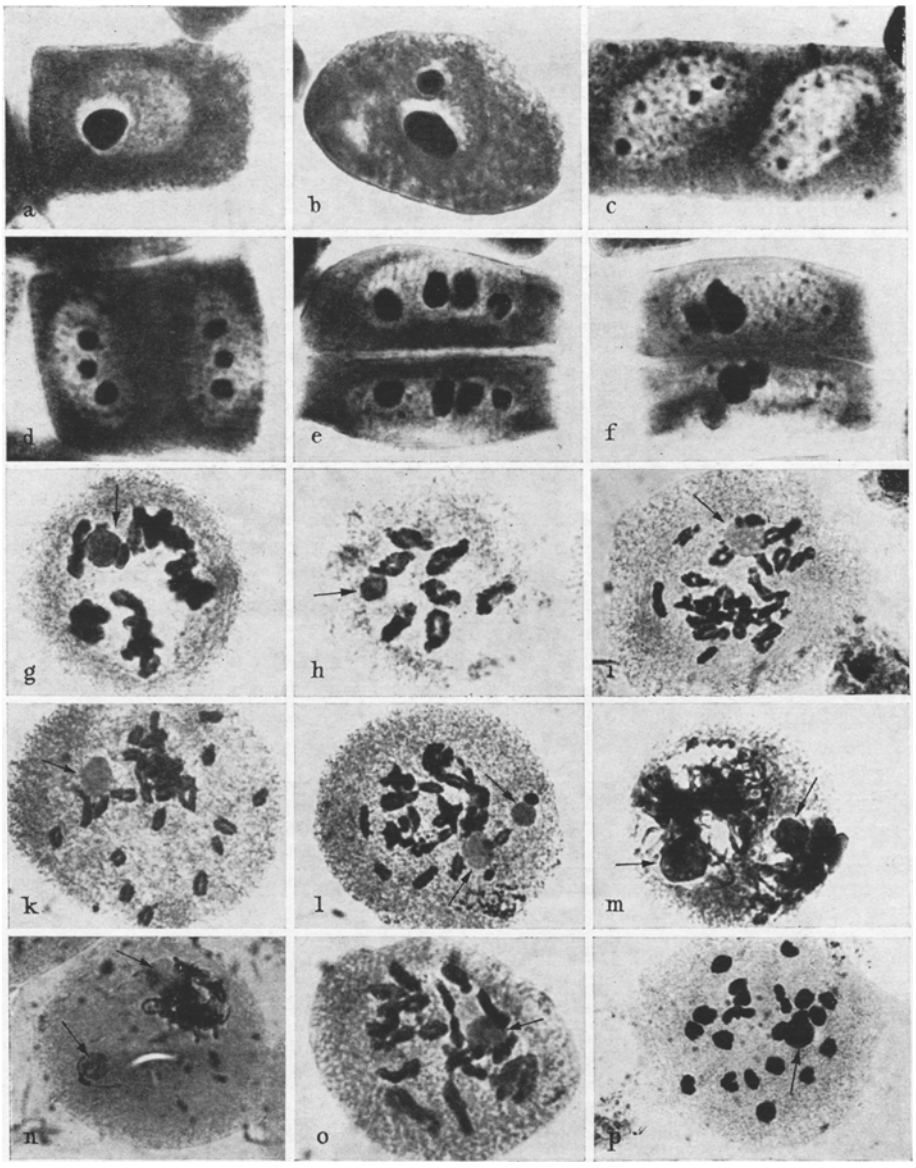

Fig. 3. Nucleolus formation in mitosis and meiosis in octoploid and hexaploid Triticale: I. Mitosis in octoploid Triticale: a) one nucleolus in interphase; b) two nucleoli in interphase; c) RNA drops in telophase; d) 3 nucleoli in telophase; e) 4 nucleoli in telophase; f) 3 nucleoli and RNA drops in telophase. — II. Meiosis in octoploid Triticale: g) one nucleolus in wheat cell; 3 chromosome pairs lying near the nucleolus; h) one nucleolus in rye cell, 1 chromosome pair lying near nucleolus; i) one nucleolus in Triticale, 4 pairs of chromosomes lying near nucleolus; k) one nucleolus in Triticale, 3 chromosome pairs lying near nucleolus; l) m) 2 nucleoli in Triticale. — III. Meiosis in hexaploid Triticale: n) 2 nucleoli in prophase; o) one nucleolus, 3 chromosome pairs lying near nucleolus; p) one nucleolus, 2 chromosome pairs lying near nucleolus

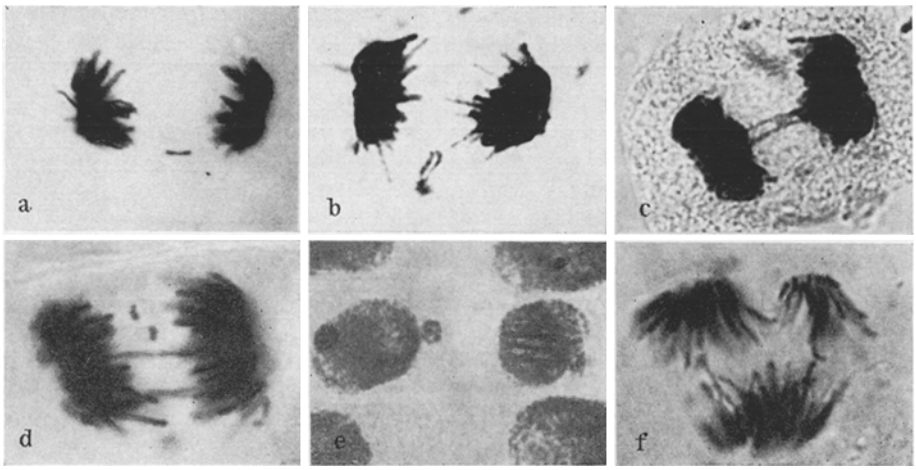

Fig. 4. Mitotic irregularities in octoploid and hexaploid Triticale: a) fragment in anaphase; b) lagging chromosome in anaphase; c) chromosome bridge; d) chromosome bridge, chromatid bridge and two fragments; e) micronuclei in telophase; f) tripolar mitosis

Table 8. *Frequency of mitotic irregularities of Triticale*

Triticale	2n	Number of studied		Frequency of abnormal cells	
		plants	cells	total	%
H34AD	56	20	1795	90	5.0 ± 0.17
31AD72	56	20	1989	119	5.9 ± 0.16
H120AD	56	20	2112	241	11.4 ± 0.22
10H120AD	56	20	1958	90	5.5 ± 0.15
H121AD	56	20	1921	127	6.6 ± 0.18
H129AD	56	20	1550	130	8.3 ± 0.22
25AD20	56	10	294	40	13.6 ± 1.99
529AD117	56	10	367	57	15.5 ± 1.88
AD from Hungary	42	22	1046	62	5.9 ± 0.7
Ad from Canada	42	9	960	74	7.7 ± 0.8
4097	42	11	550	82	14.9 ± 1.5
4139	42	25	778	64	8.2 ± 0.9
4138	42	12	527	52	9.8 ± 1.2
4209	42	15	620	39	6.2 ± 0.9
4214	42	13	439	35	7.9 ± 1.2
4223	42	11	297	33	11.1 ± 1.9
4149	42	10	522	63	12.1 ± 1.4
4217	42	10	413	62	15.0 ± 1.7

Discussion

Our results indicate that meiosis is irregular in all the Triticale studied, the frequency and type of disturbances varying from one Triticale to another. In some, the main meiotic irregularity was chromosome lagging, leading to the formation of micronuclei in the tetrads. In others, spindle formation was most disturbed, giving rise to polyads. Considerable meiotic irregularities occurred in octoploid and hexaploid Triticale. There were no differences between primary and secondary 42-chromosome Triticale. On this point contradictory opinions have been expressed in the literature. Most authors consider that meiosis is more disturbed in 56-chromosome Triticale than in 42-chromosome ones and that, consequently, hexaploid Triticale are more stable. Kiss (1966) reports that secondary Triticale are more stable. Our data do not accord with this viewpoint since up to 87.7% of aberrant tetrads were found in meiosis in the secondary Triticale of 10—12 generations. Moreover, Shulindin has derived primary Triticale with a low number of meiotic irregularities (Shulindin and Naumova, 1966). A clear-cut distinction between primary and secondary Triticale cannot, therefore, be made. The frequency and type of meiotic irregularities depends on the Triticale genotype. Pieritz (1966) has indicated that the frequency of meiotic irregularities is low in hexaploid Triticale. Out of the pollen mother cells he studied, only 5.76% had univalents and 1.97% had multivalents. However, according to our findings, the number of univalents and multivalents cannot be an adequate criterion of the frequency of meiotic irregularities in Triticale: on the one hand, chromosome pairing is not disturbed in diakinesis and, on the other hand, a small number of univalents and multivalents is found in common wheat. For instance, Riley and Kimber (1961) have established up to 6% univalents per cell, in 5 varieties of common wheat; and Zschege (1963), using spring wheat 'Heines Koga II', found up to 19.1% and 15.8% univalents and 1.7% and 0.0% multivalents per cell in 1960 and 1961, respectively. Eight years experience with 56-chromosome and 42-chromosome Triticale has led Sanchez-Monge (1958) to the conclusion that they have the same frequency of meiotic irregularity. This is in good agreement with our results. Krolow (1966) may have been wrong in considering that meiosis in hexaploid Triticale should be normal insofar as the D-genome of wheat is absent in these plants. He states that the D-genome contains specialized genes responsible for the cytological instability of Triticale, whereas the A and B genomes cannot exert a deleterious effect on meiosis. However, the absence of the D genome "per se" has no effect. This is shown by the fact that, out of the 16 different hexaploid Triticale in which meiosis was studied in the present investigation, only one hexaploid had 12% of abnormal tetrads, 9 amphidiploids had over 50% meiotic irregularities, and, 87.7% aberrant tetrads

tioned separately from that of wheat. It is likely that, in this Triticale, not all the chromosomes participate in nucleolus formation, since at anaphase of mitosis it was noted that some chromosomes were devoid of RNA. In telophase, the pronucleolar material was condensed mainly into 3 nucleoli (Fig. 3). However, in some telophases, RNA-containing drops were seen simultaneously with 3 nucleoli; a fourth nucleolus appeared sometimes through the fusion of these drops. A comparison of the size of nucleolar material in the cells with different numbers of nucleoli (Table 5) established that, at telophase with 3 nucleoli, the pronucleolar material makes up 10.5 $\pm$ 0.03 and, with 4 nucleoli, it amounts to 12.1 $\pm$ 0.05. These two estimates differ considerably; the size of the nucleolar substance is larger in the telophase with 4 nucleoli; in the telophase having RNA drops, it is 10.2 $\pm$ 0.03 and does not differ from its size in the telophase with 3 nucleoli. The data suggest that rye chromosomes do not always take part in nucleolus formation, but when the rye nucleolar organizer accumulates pronucleolar material an additional nucleolus is formed in the cell. A comparison of the actual distribution of the number of nucleoli per interphase with the theoretically expected one for 3 and 4 pairs of nucleolar organizers in the cell has shown that the actual distribution is at variance with the expected one for both 3 and 4 pairs of nucleolar organizers (Table 7). However, the actual distribution corresponds more closely with the expected one for 3 pairs of nucleolar organizers. It seems that, in the cells of this Triticale, wheat chromosomes alone were involved most frequently in the formation of the nucleoli, but sometimes rye chromosomes functioned as well. Thus, in some octoploid Triticale, the inactivation of the nucleolar organizer of rye takes place.

Table 6. *Comparison of actual distribution of the number of nucleoli per interphase with the expected distribution for 4 pairs of nucleolar organizers for Triticale 31AD72*

Number of nucleoli per interphase	1	2	3	4	5	6	7	8	χ^2
Actual distribution	281	298	169	59	10	2	1	0	
Actual distribution for 1000 cells	289	394	221	78	13.2	2.67	0.33	0	5.48
Expected distribution for 1000 cells	286	392	230	75	14.7	1.7	0.11	0.02	

$\chi^2_{0.05}$ 12.6
$\chi^2_{0.01}$ 16.8
$\chi^2_{0.001}$ 22.5

A similar position was observed in hexaploid Triticale. In most of the hexaploids studied, only 2 chromosome pairs participate in nucleolus formation. In these amphidiploids only 2 nucleoli of different size are noted occasionally (Fig. 3). Presumably, the nucleolar organizer of the rye chromosome is also inactivated. Only 3 hexaploid Triticale (4139, 4149, 5312) have one nucleolus in the formation of which 3 chromosome pairs participate; rye and wheat chromosomes are involved. It is noteworthy that these Triticale have the lowest number of meiotic irregularities (Table 1). In the other Triticale, wheat and rye genomes lying apart from each other were observed in the prophase of meiosis and lysis of part of the chromosomes took place.

It is interesting that in all the Triticale studied mitotic irregularities were found too (Table 8, fig. 4). At anaphase, in addition to lagging chromosomes, tripolar and multipolar divisions, and both chromatid and chromosome bridges (dicentric chromosomes) were found. This indicates that mutation rate is increased in the root tip cells of the amphidiploids. Mitosis, however, was less disturbed than meiosis: 5%—11.4% aberrant cells were observed in octoploids and 5.9% to 15% in hexaploids.

Table 7. *Comparison of actual distribution of number of nucleoli per interphase with the expected distribution for 4 and 3 pairs of nucleolar organizers for Triticale H121AD*

Number of nucleoli per interphase	1	2	3	4	5	6	7	8	χ^2
Expected distribution for 3 pairs of nucleolar organizers	327	410	204	51.2	6.4	0.3	0	0	
Actual distribution for 1000 cells	314.6	401	207	62.1	12.2	13.1	0	0.013	31.1
Expected distribution for 4 pairs of nucleolar organizers	298	367	275	114.8	28.6	4.3	0.35	0.01	55.8

for 3 pairs of nucleolar organizers
$\chi^2_{0.05}$ 9.5
$\chi^2_{0.01}$ 13.3
$\chi^2_{0.001}$ 18.8

for 4 pairs of nucleolar organizers
$\chi^2_{0.05}$ 12.6
$\chi^2_{0.01}$ 16.8
$\chi^2_{0.001}$ 22.5

were found in one amphidiploid. Common causes probably underlie the appearence of meiotic irregularities in hexaploid and octoploid Triticale. Perhaps the main cause of meiotic disturbances is the incompatibility between rye and wheat genomes located in one cell and the inactivation of some loci of rye chromosomes in some of the cells. In some Triticale, wheat and rye genomes are functionally united. If this occurs, all the nucleolar organizers are in an activated state. These Triticale have one common nucleolus for rye and wheat chromosomes in the prophase of meiosis, and both wheat and rye chromosomes participate in its formation. One nucleolus has been observed by Kostoff (1946) in polyploid species and in interspecies hybrids of Nicotiana. But even here some physiological disturbances in the cell interfere with normal spindle organization and multipolar cell divisions appear. This leads to the formation of aneuploid gametes and ultimately to the appearance of aneuploid plants in the progeny. In the other Triticale, rye and wheat genomes function asynchronously. Lagging of rye chromosomes during meiotic division has been mentioned in the literature (Avdulov, 1937, Vettel, 1960). Florell (1936) has indicated differences in the degree of coiling between rye and wheat chromosomes of Triticale, the rye chromosomes being more coiled. The differences in the behaviour of the chromosomes belonging to different genomes have been attributed to first, the differences in the duration of the cell cycle phases between different genomes and, second, to the incompatibility between the nucleus of one of the parent species and the cytoplasm of the other (Avdulov, 1937). Riley (1960), however, associated meiotic irregularities in Triticale with the genetic differences between rye and wheat chromosomes. From his studies of wheat lines with added rye chromosomes, Riley concluded that rye chromosomes are unable to function adequately when there is a full set of wheat chromosomes; rye chromosomes are not controlled by wheat chromosomes in the process of cell division. Our observations accord with Riley's opinion in showing that rye chromosomes are not controlled by wheat chromosomes in the process of cell division. Our studies indicate that the chromosomes of the rye genome in Triticale not only lag behind wheat chromosomes during the cell cycle, but keep apart from them in the course of meiosis. Rye chromosomes sometimes lie apart from wheat chromosomes during prophase and go through metaphase and anaphase separately. Mostly, only one wheat nucleolus functions in these Triticale. Presumably, the separation of rye chromosomes is not controlled by spindle fibres and they are distributed randomly throughout the cell.

In studies of mitosis in *Crepis capillaris*, Sidorov and Sokolov (1963) have shown that single chromosomes are no longer under the control of the whole chromosome complex and are lysed by the protoplasm. It seems that it is not just by chance that the disturbances in these Triticale consist mainly of lagging chromosomes that can be lost in the course of meiosis giving rise to aneuploids. In 12% of the case, the rye nucleolus functions with the nucleolus of wheat. In these cases rye chromosomes organize their own spindle so that rye genomes separate during meiosis and this leads to polyad formation. Perhaps there is a direct relation between the activity of the rye nucleolus and spindle fibre formation which regulates the distribution of rye chromosomes at anaphase. It has been indicated that in some plants, for instance *Crepis* hybrids, the nucleolar organizer is inactivated (Nawashin, 1934); partial inactivation of the nucleolar organizer has also been observed in the cells of *Danthonia* polyploids (DeWet, 1953). The probable reasons for the loss of the capacity of the nucleolar organizer to accumulate pronucleolar material may be of a genetic as well as physiological nature. It has been shown in maize that the lack of some chromosomes in the genome interferes with the function of the nucleolar organizer and consequently the microspores contain only discrete drops of pronucleolar substance in spite of the presence of the nucleolar chromosome (McClintock, 1934). Similar events have been observed in the premeiotic nuclei of *Lolium* under high temperature (Jain, 1957). In the cells of Triticale both causes are perhaps involved, since the interaction between rye and wheat chromosomes interferes with cell physiology.

The mutational process is enhanced in Triticale: in all the Triticale studied chromosome aberrations were found in mitosis, the majority of them being chromatid and chromosome bridges. No mutagenic agent acted on the seeds of the plants studied and the increase of naturally occurring chromosome rearrangements can be related only to the genome belonging to another plant genus. Gaul (1954) has described translocations in the somatic cells of intergeneric cereal hybrids; translocations have been observed in an amphidiploid of *Crepis rubra*×*C. foetida* by Poole (1932). An enhanced mutational process in remote hybrids has been mentioned (Karpechenko, 1935). Instability of the chromosome number is typical of hybrids between wheat and *Aegilops* and *Agropyron* (Sachs, 1952, Li and Tu, 1947), and of amphidiploids of *Nicotiana* (Yang Shung-Jun, 1964, 1965), *Avena* genus (Thomas, 1965), and the genus *Gossypium* (Bernardo, 1965). Chromosome number can sometimes be unstable in natural allopolyploids such as *Triticum aestivum* (Love, 1936) as well as in allopolyploids of the genus *Rubus* (Tompson, 1962). All the authors who have observed the instability of chromosome number have inferred that the disturbance in spindle function was its main cause. However, chimeras are found in Triticale in which it is difficult to establish the predominance of a particular chromosome number because of its variability. This is not the result of disturbed spindle formation, since we have not observed polyad

formation in meiosis in more than 30% of the total number of disturbances. Only single cells with anomalous spindles were noted in mitosis. It is most likely that aneuploid cells in Triticale arise from the asynchronized functioning of rye and wheat chromosomes and from lagging chromosomes in anaphase and telophase.

The cytological study of Triticale demonstrates that the interaction of wheat and rye genomes in the cells of one plant leads to profound derangements in cell physiology that are maintained for decades at least. Thus in Triticale Rimpau produced in 1889, irregularities in both meiosis and mitosis are noted which are identical with those found in Triticale derived only recently. Differences between octoploid and hexaploid Triticale were not observed in this respect.

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