Theor Appl Genet (1983) 66:159–167

Origin of nuclear aberrations and seed shrivelling in triticale: a re-evaluation of the role of C-heterochromatin

J. P. Varghese* and T. Lelley

Institut für Pflanzenbau und Pflanzenzüchtung, Georg-August-Universität, von-Siebold-Straße 8, D-3400 Göttingen, Federal Republic of Germany

Received March 11, 1983
Communicated by H. F. Linskens

Summary. The effect of C-heterochromatin on the origin of nuclear aberrations and seed shrivelling was investigated in four triticale lines, each consisting of a pair of genotypes designated A (producing plump, well-filled seeds) and B (with shrivelled seeds). The relative DNA content in the polyploid nuclei of endosperms, 42 h after pollination, was estimated by Feulgen cytophotometry. The observed frequency of polyploid nuclei, 0.85% and 5.69%, respectively, in the two genotypes 1A and 1B caused a reduction in nuclear number of 3.27% and 18.54% at this stage of development. In the B genotype, producing shrivelled grains, polyploidisation started earlier than in the A genotype. An examination of the Giemsa karyotype of the mitotic chromosomes of the rye genome in the four triticale pairs revealed no considerable differences in the banding pattern between the A and B genotypes. Giemsa staining of endosperms, 2–3 days after pollination, clearly showed that bridges without bands, most probably involving wheat chromosomes, were also present. An experiment designed to simulate spindle disturbances in developing endosperms by colchicine treatment revealed that polyploid nuclei can be formed by spindle malfunctions as well.

Key words: C-heterochromatin – Polyploid nuclei – Seed shrivelling – Triticale

Introduction

In triticale, various types of nuclear aberrations occur during the coenocytic stage of endosperm development (Moss 1970; Kaltsikes 1973; Bennett 1974, 1977; Kaltsikes and Roupakias 1975; Kaltsikes et al. 1975). Bridges were reported to be a major class of aberrants observed during this stage, and they were thought to be formed by the non-separation of the chromatids at their telomeres (Bennett 1974, 1977). Lima-de-Faria and Jaworska (1972) and Verma and Rees (1974) found late replicating C-heterochromatin in the telomeres of rye chromosomes. Coenocytic endosperms, selectively stained with Giemsa, indicated that bridges were formed by rye chromosomes. Based on this observation (Bennett 1977) suggested that the late-replicating C-heterochromatin in the telomeres of rye chromosomes might be the major cause of bridging subsequently leading to the formation of aberrant polyploid nuclei. A new breeding strategy for triticale, namely the utilisation of structurally modified rye chromosomes with less C-heterochromatin, therefore, has been advocated by him (Bennett 1977, 1981). Gustafson et al. (1980), Gustafson and Bennett (1982) and Schlegel (1980) have supported this proposal. But contradicting results have also been reported by Lukaszewski and Apolinarska (1981) and Pilch (1981 a, b).

The frequency of aberrant nuclei is correlated to the degree of shrivelling at maturity, irrespective of the mechanism of their origin. The polyploid nuclei represent the most frequent class of aberrations (Kaltsikes and Roupakias 1975; Kaltsikes et al. 1975). They contain large amounts of DNA depending on the level of ploidy, and their subsequent disintegration represents the loss of a higher number of nuclei. Thomas et al. (1980) suggested that the mere frequency of these nuclei is not a good measure of the degree of aberrancy caused by them.

The present investigation was undertaken to determine to what extent the formation and disintegration of

* Present address: Lecturer in Botany, C.M.S. College, Kottayam 686001, Kerala, India

polyploid nuclei reduce the potential number of endosperm cells and to re-examine the role of heterochromatin in nuclear aberrations and seed shrivelling.

Materials and methods

Four pairs of triticale genotypes, each pair consisting of an A genotype (producing plump, well-filled seeds) and a B genotype (with shrivelled seeds) generated by disruptive selection for shrivelling, were used in this investigation (Table 1).

Giemsa staining of root tip cells and endosperms was performed according to Gustafson et al. (1976).

Cytophotometric measurement of the relative amount of DNA (McLeish and Sunderland 1961; Miksche 1971; Bennett and Smith 1976) in polyploid nuclei was made 42 h after pollination in 10 endosperms each of the genotypes 1A and 1B. The ovaries were hydrolysed with 5N HCl at room temperature for 10 min and the embryo sacs were dissected out. A cold hydrolysis was preferred in order to prevent excessive hydrolysis of DNA (Nagl personal communication). The ensuing Feulgen staining procedure was carried out as described by Varghese et al. (1983). Light absorption at 570 nm by the nuclei was measured using a Zeiss Scanning Photometer Microscope (100× objective) with computer PDP 12 and a programme designated APAMOS (Automatic Photometric Analysis of Microscopic Objects through Scanning; Zeiss APAMOS Version 2 (1973); Zimmer (1974). The absorption of 3n=6C nuclei was used as the standard to calculate the ploidy level of the various nuclei measured.

In order to simulate spindle disturbances during endosperm development, ovaries, 6 h after pollination, were treated with a 0.05% solution of colchicine by the tongue slit method (Röbbelen 1972). A median incision was made in the top internode with a blade, about 4 cm below the base of the ear and extended downwards for 6 cm and cut off in its lowest part in such a way that a tongue was formed. This was introduced into a vial (6.5 cm × 1 cm diameter) filled with the colchicine solution which was fastened to the culm by a cellophane tape. The top of the vial was closed with cotton. In this way the colchicine was transported along with the transpiration stream into the embryo sacs without separating the ear from the plant. The treated ears were fixed 42 h later, i.e. 48 h after pollination.

Results

Number of polyploid nuclei in relation to seed shrivelling

Polyploid nuclei represent the most frequent class among the various types of aberrations appearing during the coenocytic stage (Table 2). A highly significant negative correlation of their frequency in the endosperms of different genotypes with the grain quality score and thousand grain weight has been calculated from the material ($r=-0.88$ and $r=-0.87$, respectively; $P=0.01$) for the year 1981. The same trend also was visible in 1982, although here the value of the correlation coefficient only approached significance ($r=-0.66$ and $r=-0.63$, respectively; $P=0.05$).

DNA content in polyploid nuclei estimated by Feulgen-cytophotometry

The relative DNA content in the polyploid nuclei in ten endosperms each, 42 h after pollination, in one pair of genotypes, viz. 1A and 1B, was cytophotometrically estimated (Table 3). Light absorption by all 10 polyploid nuclei present in the endosperms of 1A, and 49 out of 59 in 1B were measured in addition to a total of 64 normal (3C or 6C) triploid nuclei as control.

The average absorption of a normal 3n=6C endosperm nucleus was used as the standard to calculate the ploidy levels of all nuclei measured. A calculated ploidy level of 2 means that the nucleus actually occupies a ploidy level of 6n, a calculated ploidy level of 4 indicates an actual ploidy level of 12, etc. This further indicates that these nuclei contain an amount of DNA equivalent to 2 and 4 normal triploid nuclei, respectively. Nuclei whose ploidy levels were not in regular multiples of the 3n=6C absorption value were considered to be in transition between ploidy levels. The amount of DNA present in all polyploid nuclei measured was calculated in terms of the equivalent number of normal triploid nuclei.

Table 3 shows that the 10 polyploid nuclei measured in genotype 1A contained an amount of DNA equivalent to 39.5 normal triploid nuclei. One poly-

Table 1. Grain characteristics of the 8 genotypes used. All plants were grown under greenhouse conditions of 16 h light and 20±3 °C. The values represent the mean (± SE) of two consecutive years.

Entry	Genotypes	Grain quality score (1–9)[a]	LSD 1%	Thousand grain wt (g)	LSD 1%
AD 206	1 A	5.18±0.14		60.5±2.35	
	1 B	3.85±0.15	0.53	44.6±1.82	8.72
6 TA 313 × Kiss 028 M_1	2 A	4.19±0.16		41.2±2.13	
	2 B	2.33±0.11	0.54	25.4±1.32	7.30
Kiss 028 M_1 × 6 TA 313	3 A	5.11±0.23		51.4±1.86	
	3 B	4.27±0.16	0.77	50.6±2.52	7.33
Tr 6 TA 313 (*timopheevi* cytoplasm)	4 A	4.50±0.22		53.9±1.45	
	4 B	3.65±0.13	0.80	37.0±2.59	8.41

[a] The score scales from 1 = shrivelled to 9 = well filled seeds

Table 2. Frequency of polyploid nuclei (per 1000) in relation to grain quality score (Table 1) and thousand grain weight (TGW)

Genotype	1981			1982		
	Polyploid nuclei	Grain quality score	TGW g	Polyploid nuclei	Grain quality score	TGW g
1 A	7.47	5.0	65.0	5.55	5.4	57.3
1 B	14.08	3.5	44.0	8.57	4.4	44.8
2 A	20.21	3.7	36.4	4.82	4.7	47.3
2 B	38.89	2.3	23.6	12.41	2.4	27.6
3 A	6.95	5.8	51.6	9.50	4.7	51.2
3 B	14.21	4.6	50.8	8.44	4.2	50.6
4 A	8.75	4.4	53.8	5.23	4.8	54.7
4 B	17.39	3.4	32.4	16.37	3.9	42.8

Correlation (*, ** $P = 0.05$ and 0.01, respectively) between:
frequency of polyploid nuclei and grain quality score $r(1981) = -0.88^{**}$
$r(1982) = -0.66$
frequency of polyploid nuclei and thousand grain weight $r(1981) = -0.87^{**}$
$r(1982) = -0.63$

ploid nucleus, therefore, contained on an average 3.95 triploid nuclei. Likewise, the 49 polyploid nuclei measured in 1B accounted for 184.63 triploid nuclei, one polyploid nucleus being equivalent to 3.77 triploid nuclei. These numbers represent a reduction in the mitotic population amounting to 3.27% and 18.54% in 1A and 1B, respectively.

Maximum ploidy level and stage of initiation of polyploidy

The developmental stage, i.e. the nuclear division cycle after fertilisation in which polyploidisation began was calculated on the basis of the maximum level of ploidy measured in the two genotypes. The maximum absorp-

Table 3. Estimated loss of normal endosperm nuclei caused by polyploid nuclei

Genotype	No. of			Ploidy level (in multiples of 3n)							Total loss in the no. of 3n nuclei	Reduction in nuclear no. (%)
	Total endosperm nuclei	Polyploid nuclei present		2	3	4	5	6	13	Transition ploidy levels		
1 A	1180	10	No. of polyploid nuclei measured		1	1	1			7[a]		
			Corresponding loss in 3n nuclei		3	4	5			27.5	39.5	3.27
1 B	860	59[c]	No. of polyploid nuclei measured	5	6	3	1	1	1	32[b]		
			Corresponding loss in 3n nuclei	10	18	12	5	6	13	120.63	184.63	18.54

[a] Transition ploidy levels ranging between 1 and 5 times 3n
[b] Transition ploidy levels ranging between 1 and 14.44 times 3n
[c] Only 49 nuclei could be measured

Table 4. Maximum ploidy level measured in endosperms, 42 h post-pollination, (based on the absorption of light at 570 nm by normal and polyploid nuclei) and the number of division cycles required to reach this level

Genotype Absorption Nucleus	1 A		1 B	
	Average	Maximum	Average	Maximum
	Normal 3n = 6C	Polyploid	Normal 3n = 6C	Polyploid
Measured absorption	5135	25928	4972	71809
Calculated ploidy level		5.05 (= 30, 30C)		14.44 (= 86, 64C)
No. of division cycles		2.26		3.80

tion measured in a polyploid nucleus in 1A was 25928 and the average absorption of a 3n nucleus, 5135 (Table 4). That means that this nucleus occupied a ploidy level of 5.05 times 3n and had a C value of 30.3. Calculations show that 2.26 nuclear division cycles were required to reach this ploidy level since polyploidisation began. In the same way, the maximum ploidy level in 1B was calculated to be 14.44 times 3n with a C value of 86.64. To reach this level of ploidy, 3.8 nuclear division cycles were required.

Endosperm development during the early to mid coenocytic stage follows an exponential increase in nuclear number. Figure 1 shows the increase in the number of nuclei till the 7th nuclear division cycle after fertilisation. The approximate stage in which polyploidisation started was estimated from the average number of endosperm nuclei present and the maximum ploidy level reached at this stage of development. For example, the average number of endosperm nuclei in 1B was 86. From the point corresponding to this number in the y-axis in Fig. 1, the nuclear division cycle in which polyploidisation started can be determined. As the diagram shows, in the case of 1B it began 3.8 division cycles before this point. That corresponds to the 4–8 nuclear stage or the third nuclear division after fertilisation. In the same way, it was estimated that in 1A polyploidisation began in the 16–32 nuclear stage or the 5th nuclear division cycle after fertilisation.

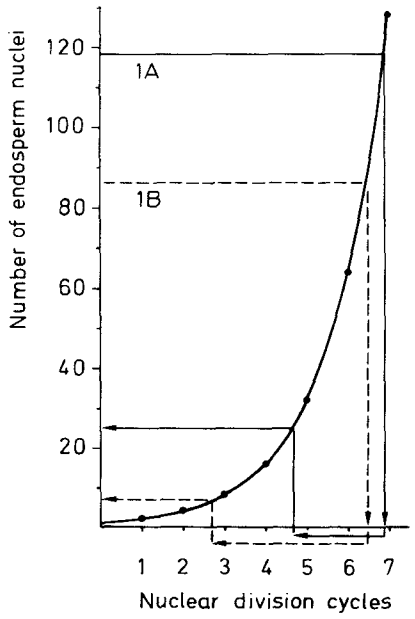

Fig. 1. Stage of initiation of the first polyploid nucleus in genotypes *1A, 1B*

It is important to note that polyploidisation in 1B, producing shrivelled grains, started much earlier than in 1A.

Comparison of Giemsa karyotypes

The Giemsa karyotype of rye chromosomes from root tip cells was studied (Fig. 2) in order to determine differences in the banding pattern between the A and B genotypes. The Giemsa bands were visually compared within the genotype pairs. A secondary band is present on the long arm (upper arm in the picture) of chromosome 2R in the case of genotype 1B, whereas it is absent in the corresponding chromosome in 1A. The telomeric band on the short arm in this chromosome (2R) and an intercalary band on chromosome 6R appear to be larger in 1B in comparison to the respective ones in 1A. Comparison of genotypes 3A and 3B reveals that an intercalary band present on the long arm of chromosome 6R is larger in 3A than in 3B. Since the differences in the number and size of C-bands, especially at the telomeres, of A and B genotypes are negligible, they can not be held responsible for the difference in grain shrivelling in these genotypes. The disruptive selection for shrivelling was unaccompanied by any considerable change in the Giemsa banding pattern.

Giemsa stained bridges without bands

Endosperms of the genotype 2B were stained with Giemsa 2–3 days after pollination to see whether chromatid bridges without Giemsa bands are present. Figure 3 shows two nuclei connected by three bridges. A major band is seen on the upper bridge, minor ones on the lower bridge and no bands on the middle one. The usually observed Giemsa bands are visible in the two nuclei. On three out of a total of seven bridges observed, no bands were present. The bridges without

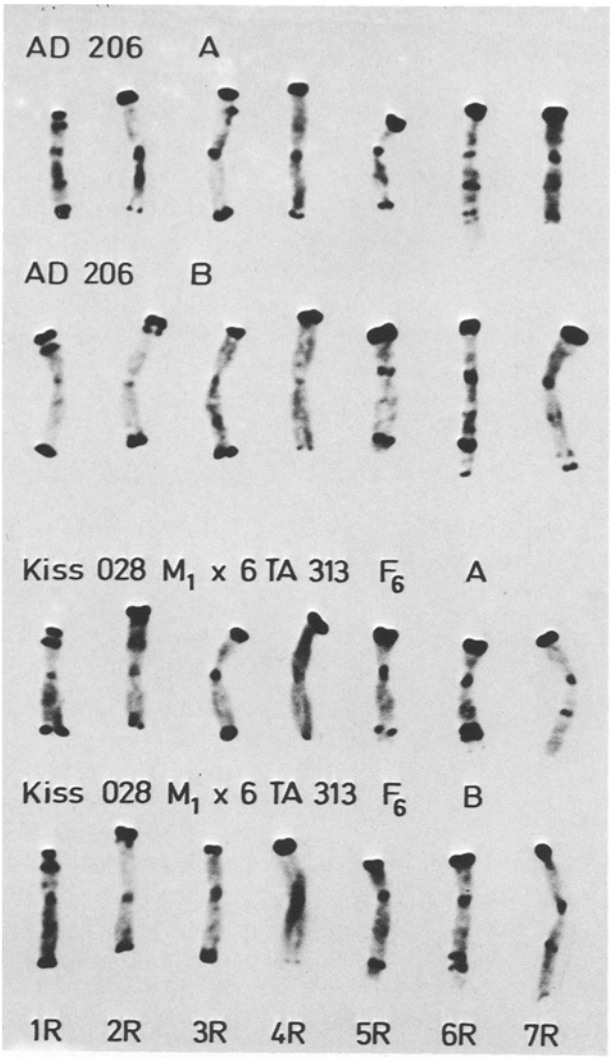

Fig. 2. Giemsa-karyotypes of the genotypes 1A, 1B (*AD 206 A,B*) and 3A, 3B (*Kiss 028 $M_1 \times 6$ TA 313 F_6 A, B*)

bands are considered to be formed from wheat chromosomes.

Effect of colchicine on endosperm mitosis

An experiment was conducted to simulate spindle disturbances during early endosperm development. Endosperms 6h after pollination were treated with a 0.05% solution of colchicine and fixed 42 h thereafter. The following three observations were made on such treated endosperms. (1) More than 50% of the endosperms examined contained huge polyploid nuclei only (Fig. 4). (2) These nuclei resembled polyploid nuclei observed in control endosperms (Fig. 5). (3) The number of nuclei in treated endosperms was considerably less than that in control endosperms fixed at the same time (Table 5).

Discussion

Polyploid nuclei in relation to shrivelling

Thomas et al. (1980) reported a positive correlation between the frequency of polyploid nuclei and the associated degree of seed shrivelling in triticales (calculated from the data of Kaltsikes et al. 1975) and in wheat-rye addition lines (calculated from the data of Kaltsikes and Roupakias 1975). This close correlation also holds true in the case of the present material. The polyploid nuclei constituted, in all genotypes examined, with one exception, the most frequent class of aberrants, as reported by Kaltsikes and Roupakias (1975) and Kaltsikes et al. (1975). They undergo cycles of DNA duplication in pace with the neighbouring nuclei without separation of the products. Each of these nuclei carries a multiple amount of DNA of a normal triploid

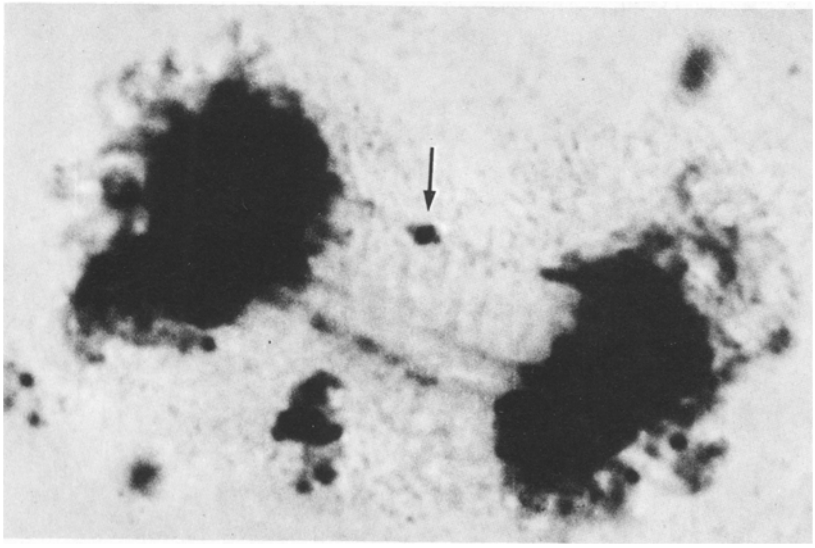

Fig. 3. Two endosperm nuclei in the genotype 2B connected by 3 bridges. A major Giemsa-band is seen only on one bridge (*arrow*) (×800)

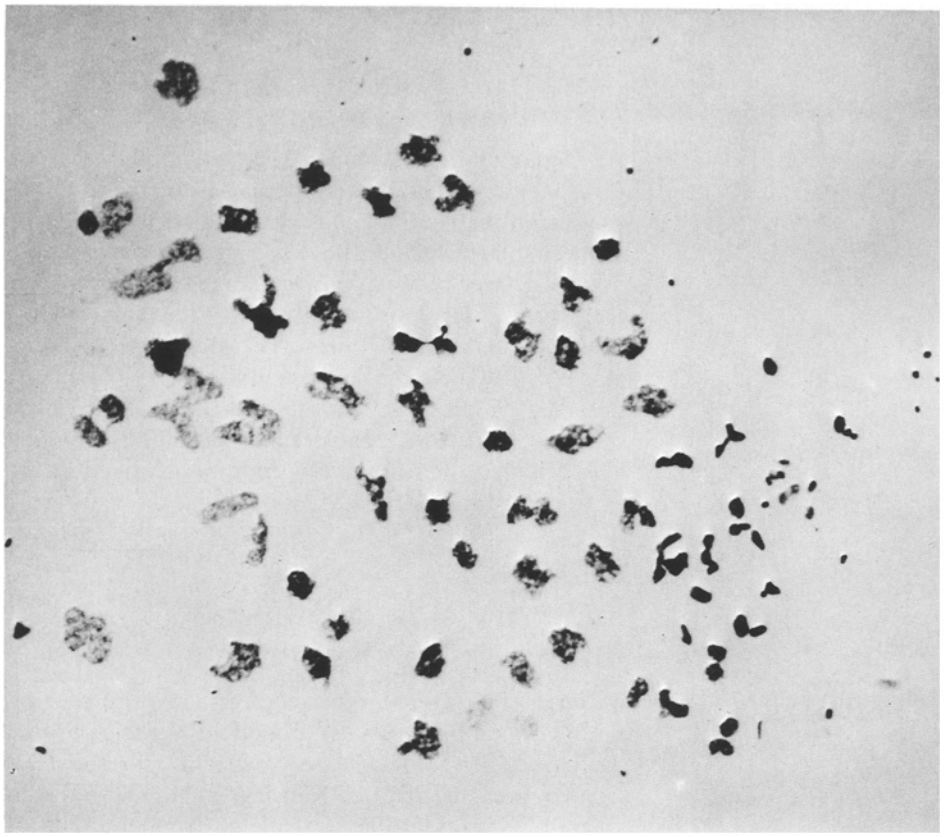

Fig. 4. An endosperm of 2B after treatment with colchicine, 48 h postpollination, containing only polyploid nuclei (×20)

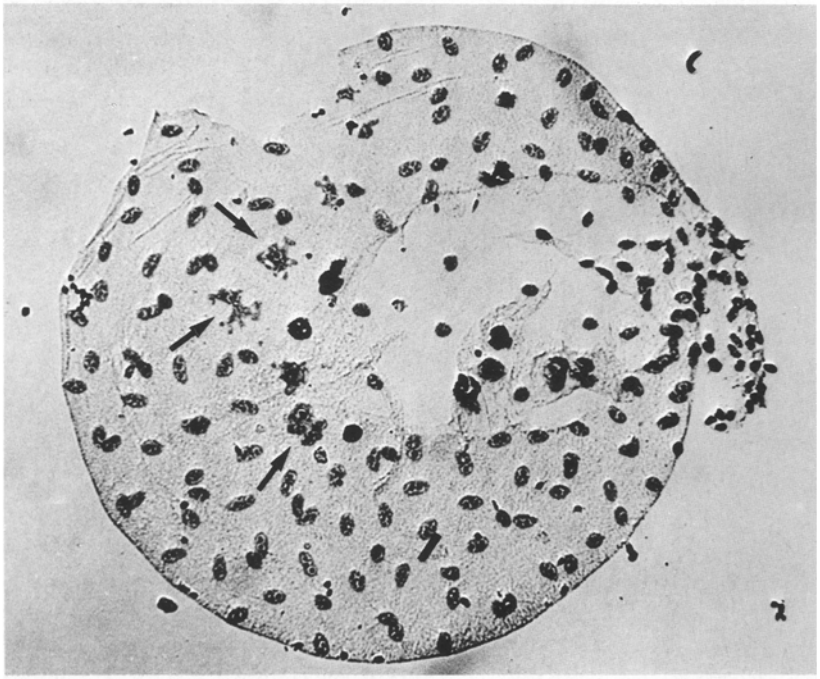

Fig. 5. An endosperm (untreated) of 2B, 48 h post-pollination (×20). Polyploid nuclei are indicated with *arrow*.

Table 5. Mean number (± SD) of nuclei in endosperms treated with colchicine, 48 h after pollination

Genotype	Mean no. of nuclei	
	Treated endosperms $n=10$	Control endosperms $n=5$
1 A	41.9±4.97	183.0± 12.73
1 B	19.4± 1.93	140.6± 21.23
2 A	33.0± 3.67	283.4± 18.51
2 B	58.6± 2.77	100.8± 10.31

nucleus, depending on the number of nuclear division cycles elapsed since polyploidisation began. Consequently, the later disintegration of each of these nuclei represents the loss of a higher number of normal endosperm nuclei.

It has been suggested that the mere frequency of polyploid nuclei is not a meaningful measure of the degree of aberrancy caused by them (Thomas et al. 1980). Expressing their frequency in percentage of the total nuclei present does not reveal their level of ploidy i.e. to what extent (in terms of the number of nuclei or cells) they effectively reduce the endosperm cell number.

Cytophotometric measurement of the relative DNA content of polyploid nuclei in the endosperms of the genotypes 1A and 1B showed that their observed frequency of 0.85% and 5.69% in the two genotypes, respectively, actually reduced the potential number of nuclei by 3.27% and 18.54% 42 h after pollination (Table 3). Kaltsikes et al. (1975), based on four assumptions calculated that a reduction of 18.3% to 20.3% in the mitotic population was caused by the polyploid nuclei between the 9th and 14th divisions after fertilisation (i.e. at a later stage of development). The results of the present investigation show that a reduction in the potential number of cells can be caused by these nuclei even at a much earlier stage of development.

The process of polyploidisation was found to be initiated in 1B much earlier, i.e. in the 3rd division cycle after fertilisation, when compared to 1A (Fig. 1), where it began only in the 5th division cycle. In conclusion, the earlier the origin of a polyploid nucleus, the greater is the damage caused by way of reduction of the potential number of endosperm cells. This is in accordance with the findings of Bennett (1974).

Heterochromatin in relation to shrivelling

Bennett (1974, 1977) proposed a hypothesis of a causal chain, linking telomeric C-heterochromatin of rye chromosomes to the frequency of aberrant nuclei produced during the coenocytic stage and ultimately to the degree of seed shrivelling at maturity. This hypothesis contends that nuclear bridging is invariably caused by rye chromosomes by virtue of their telomeric heterochromatin. In this context the following points are worth re-examining:

(1) Sister chromatid exchange (SCE) has been demonstrated to be a common feature of mitosis and occurs with a higher frequency than chromosome aberrations (Perry 1980). Such a SCE followed by an erroneous reunion of the free chromosome ends could also be responsible for bridge formation in triticale. An indication of this mitotic error is the appearance of fragments of varying size, as seen by Moss (1970) and also observed in the present material. U type exchange leading to bridge formation was frequently observed in the meiosis of rye inbred lines (Jones and Brumpton 1971; Lelley, unpublished). This type of a bridging mechanism is totally independant of the presence or absence of telomeric heterochromatin.

(2) It is not yet clear whether only rye chromosomes are responsible for bridging or whether wheat chromosomes could be involved as well. A bridge formed by the non-separation of telomeric heterochromatin, when stained with Giemsa should show the Giemsa band in the centre or at least in the central portion of the bridge. Eleven of the 14 rye telomeres possess major Giemsa bands and the other three, at least minor bands (Darvey and Gustafson 1975; Merker 1975). Therefore, a bridge showing distinguishably large major or minor bands in its central portion may be identified as being formed of rye chromosomes and those without any bands probably of wheat chromosomes.

Bridges with and without Giemsa bands have been observed in the present material. The middle bridge in Fig. 3 for example can be identified as being formed from a wheat chromosome in contrast to the upper and lower ones carrying C-band. Thus obviously bridges are not exclusively formed of rye chromosomes by virtue of their telomeric heterochromatin in the present material. Moreover, in Feulgen stained preparations anaphase cells with more than 14 bridges were found which necessarily include wheat chromosomes.

(3) Spindle disturbances might be considered as a third cause of mitotic failure. Moss (1970) observed 73% of the aberrant nuclei in developing endosperms of *Triticum aestivum* × *Secale ancestrale* hybrids to be caused by spindle faults.

Our experiment, designed to simulate spindle disturbances in developing endosperms, demonstrates clearly that spindle malfunction or disturbances can result in the formation of polyploid nuclei. Thus, the possibility of the occurrence of such spindle disturbances in the coenocytic stage and its contribution to the formation of aberrant nuclei can not be ruled out.

Apart from the above question of the role of rye chromosomes, the evidence of a direct relationship between the presence of C-bands and kernel shrivelling in triticale is also contradictory (Thomas et al. 1980). The loss of heterochromatic bands from the long arm of 7R and the short arm of 6R, taken singly, compared in isogenic backgrounds, did not have any effect on shrivelling (Merker, personal communication; Kaltsikes, personal observation, quoted in Thomas et al. 1980). Thomas et al. 1980 reported that the triticale line 6A 250 and the cultivar 'Beagle' produced better seeds although they contained more heterochromatin than comparable varieties, viz. 6A 190 and 'Driaria', respectively (which contained less heterochromatin but produced more shirvelling). In the four genotype pairs which we have examined by the Giemsa banding technique, there were no major differences within members of individual pairs with respect to the Giemsa bands (Fig. 2), although they differed significantly in seed shrivelling. These results also do not support the assumption of a causal relationship between C-heterochromatin and seed shrivelling.

A new breeding strategy has been proposed by Bennett (1977, 1980, 1981) and Gustafson et al. (1980) to improve kernel shrivelling in triticale, namely controlled reduction of telomeric heterochromatin from rye chromosomes and the use of such modified rye chromosomes for further breeding. Bennett (1977) compared the C-banding pattern of seven rye addition lines to hexaploid wheat with their effects on the production of aberrant nuclei. Chromosomes III and VI of rye were modified by a terminal deletion in the short arm (Singh and Röbbelen 1976). It was observed that the chromosomes with the deletions produced significantly less number of aberrants than the ones without the deletion. The effect of modified rye chromosomes on yield and kernel shrivelling has been tested in replicated field trials and improvement of these characters have been reported (Bennett 1981). Gustafson and Bennett (1982), Bennett and Gustafson (1982) and Zillinsky (1980) have also reported similar results.

This strategy lays emphasis on structural alterations of chromosomes (for the controlled reduction of telomeric heterochromatin of rye chromosomes) disregarding the natural genic variability available as a source for the improvement of triticale. The production of genetic variability by conventional breeding methods and the selection of better types from the spectrum of variability produced offers a simple and better way of dealing with the problem rather than screening for or trying to induce structural changes in rye chromosomes. Moreover, even if the new strategy succeeds in developing improved triticale lines with specific nucleotypic characters they would lead to a strong narrowing down of the genetic base of the crop.

May and Appels (1980) using in situ hybridisation techniques for the identification of translocated chromosomes of wheat and rye showed that loss of telomeric heterochromatin is due to the loss of the entire arms of rye chromosomes. They also suggested that it is the addition of wheat euchromatin rather than the removal of rye heterochromatin that accounts for the favourable performance of modified rye chromosomes. More recently, Pilch (1981a) investigated the rye chromosome constitution and the presence of telomeric heterochromatin in these chromosomes in 26 most widely and 24 most narrowly adapted triticale strains from the 10th International Triticale Screening Nursery of CIMMYT. He reported that 85% of the most widely adapted lines possessed all the seven rye chromosomes, accumulated fewer modified rye chromosomes and contained more telomeric heterochromatin in the rye chromosomes in comparison to the 24 most narrowly adapted strains, 96% of which had various R/D substitutions. Similar results were also obtained by the same author using a different set of lines (Pilch 1981b). Lukaszewski and Apolinarska (1981) screened 83 advanced and 7 unselected winter triticale lines from different initial crosses for the presence of rye chromosomes. They reported that 76 out of 83 advanced lines had all the seven rye chromosomes, suggesting a positve selection pressure favouring the retention of the full rye genome. Their report also suggests that not only the full complement of rye chromosomes, but also their natural structure may be necessary for a better functioning of winter triticales.

The results of the present investigation, corroborating previously published reports do not permit us to conclude that telomeric C-heterochromatin of the rye chromosomes is responsible for causing seed shrivelling in triticale. It is not karyotypic criteria but such factors as reduced cell division rate, cell number and disturbances during endosperm mitosis that contribute to the production of shrivelled grains (Varghese et al. 1983) as expressions of an imbalanced genotype. In order to improve grain quality in triticale a more systematic use of the available genotype variation, of wheat and (more important) of rye should be given priority.

Acknowledgements. We are grateful to Prof. Dr. Dr. G. Röbbelen for his continued interest in this work and for critically reading the manuscript. – Prof. Dr. V. Neuhoff and Dr. H. G. Zimmer, Dept. of Neurochemistry, Max Planck Institute of Experimental Medicine, Göttingen, kindly made the facilities of their laboratory available for carrying out the cytophotometric measurements of the endosperms. – We thank Mrs. N. Hoffmann for her excellent technical assistance in Giemsa-staining.

This investigation was financially supported by the Deutsche Forschungsgemeinschaft. The first author gratefully acknowledges a scholarship offered to him by the Ökumenisches Studienwerk e.V., Bochum, for his doctoral work, of which this paper forms a part.

References

Bennett MD (1974) Meiotic, gametophytic and early endosperm development in triticale. In: MacIntyre R, Campbell M (eds) Triticale: Proc Int Symp, El Batan, Mexico. Int Dev Res Center, Ottawa, Canada, IDRC-024e, pp 137–148

Bennett MD (1977) Heterochromatin, aberrant endosperm nuclei and grain shrivelling in wheat-rye genotypes. Heredity 39:411–419

Bennett MD (1980) Theoretical and applied DNA studies and triticale breeding. In: Proc Meeting Cereal Sect of EUCARPIA on Triticale. Hodowla Roślin Aklimatyzacja I. Nasiennictwo 24:289–297

Bennett MD (1981) Nuclear instability and its manipulation in plant breeding. Philos Trans R Soc London, Ser B 292: 475–485

Bennett MD, Gustafson JP (1982) The effect of telomeric heterochromatin of *Secale cereale* on triticale (× *Triticosecale*). 2. The presence or absence of blocks of heterochromatin in isogenic backgrounds. Can J Genet Cytol 24: 93–100

Bennett MD, Smith JB (1976) Nuclear DNA amounts in angiosperms. Proc R Soc London, Ser B 274:227–274

Darvey NL, Gustafson JP (1975) Identification of rye chromosomes in wheat-rye addition lines and triticale by heterochromatin bands. Crop Sci 15:239–243

Gustafson JP, Bennett MD (1982) The effect of telomeric heterochromatin from *Secale cereale* on triticale (×*Triticosecale*). 1. The influence of the loss of several blocks of telomeric heterochromatin on early endosperm development and kernel characteristics at maturity. Can J Genet Cytol 24:83–92

Gustafson JP Bennett MD, Larter EN (1980) Rye chromosome substitutions and modifications in 42-chromosome triticales. In: Proc Meeting Cereal Sect of EUCARPIA on Triticales. Hodowla Roślin Aklimatyzacja I. Nasiennictwo 24:373–382

Gustafson JP, Evans LE, Josifek K (1976) Identification of chromosomes in *Secale montanum* and individual *S. montanum* additions to 'Kharkov' wheat by heterochromatin bands and chromosome morphology. Can J Genet Cytol 18:339–343

Jones GH, Brumpton RJ (1971) Sister and non-sister chromatid U-type exchange in rye meiosis. Chromosoma 33: 115–128

Kaltsikes PJ (1973) Early seed development in hexaploid triticale. Can J Bot 51:2291–2300

Kaltsikes PJ, Roupakias DG (1975) Endosperm abnormalities in *Triticum-Secale* combinations. 2. Addition and substitution lines. Can J Bot 53:2068–2076

Kaltsikes PJ, Roupakias DG, Thomas JB (1975) Endosperm abnormalities in *Triticum-Secale* combinations. 1. ×*Triticosecale* and its parental species. Can J Bot 53: 2050–2067

Lima-de-Faria A, Jaworska H (1972) The relationship between chromosome size gradient and the sequence of DNA replication in rye. Hereditas 70:39–58

Lukaszewski AJ, Apolinarska B (1981) The chromosome constitution of hexaploid winter triticales. Can J Genet Cytol 23:281–285

May CE, Appels R (1980) Rye chromosome translocations in hexaploid wheat: a re-evalution of the loss of heterochromatin from rye chromosomes. Theor Appl Genet 56: 17–23

McLeish J, Sunderland N (1961) Measurement of deoxyribonucleic acid (DNA) in higher plants by Feulgen photometry and chemical methods. Exp Cell Res 24:527–540

Merker A (1975) Chromosome composition of hexaploid triticale Hereditas 80:41–52

Miksche JP (1971) Intraspecific variation of DNA per cell between *Picea sitchensis* (Bong.) Carr. Provenances. Chromosoma 32:343–352

Moss JP (1970) Endosperm failure and incompatibility in crosses between *Triticum* and *Secale*. Chromosomes Today 3:124–132

Perry PE (1980) Chemical mutagens and sister chromatid exchange. In: Serres FJ de, Hollaender A (eds) Chemical mutagens, vol VI. Plenum, New York, pp 1–39

Pilch J (1981a) analysis of the rye chromosome constituion and the amount of telomeric heterochromatin in the widely and narrowly adapted triticales. Theor Appl Genet 60: 145–149

Pilch J (1981b): Rye chromosome constitution and the amount of telomeric heterochromatin of the widely and narrowly adapted CIMMYT hexaploid triticale. Z Pflanzenzücht 87:58–68

Röbbelen G (1962) Zur Mutagenität von Urethanen. Z Vererbungsl 93:256–263

Schlegel R (1980) Amphidiploid hybrids from crosses of hexaploid wheat with several species of rye and the relationship between the amount of heterochromatin and meiotic chromosome pairing. In: Proc Meeting Cereal Sect of EUCARPIA on Triticale. Hodowla Roślin Aklimatyzacja I. Nasiennictwo 24:307–314

Singh RJ, Röbbelen G (1976) Giemsa banding technique reveals deletions within rye chromosomes in addition lines. Z Pflanzenzücht 76:11–18

Thomas JB, Kaltsikes PJ, Gustafson JP, Roupakias DG (1980) Development of kernel shrivelling in triticale. Z Pflanzenzücht 85:1–27

Varghese JP, Lelley T, Röbbelen G (1983) Cell division rate, cell number and nuclear aberrations in relation to seed shrivelling in triticale. Z Pflanzenzücht (in press)

Verma SC, Rees H (1974) Giemsa staining and the distribution of heterochromatin in rye chromosomes. Heredity 32: 118–122

Zeiss Scanning Microscope Photometer with Computer PDP 12. Software APAMOS version 2, 1973

Zillinsky FJ (1980) The influence of chromosome substitutions on some agronomic characteristics of hexaploid triticale. In: Proc Meeting Ceral Sect of EUCARPIA on Triticale. Hodowla Roślin Aklimatyzacja I. Nasiennictwo 24: 383–388

Zimmer HG (1974): Microskopie mit programmgesteuerter photoelektrischer Objektabtastung und Bildauswertung zum Zwecke der Krebsvorsorgeuntersuchung. Bundesministerium für Bildung und Wissenschaft: Studie Physikalische Technologie. BMBW-FB 1-40