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Nuclear Instability and its Manipulation in Plant Breeding

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Nuclear instability and its manipulation in plant breeding

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Nuclear instability occurs spontaneously in a typically very small proportion of cells of every individual, even in crop varieties. Of greatest interest to the cereal breeder are instabilities in the germ line, which produce off-types among progeny, or in the endosperm, which reduce grain quality.

Nuclear instabilities in crop plants merit cytological investigation for several reasons: first, to ensure that biologically possible standards of genetical purity are set for varieties in agriculture; secondly, because once understood, nuclear instability may be usefully applied in plant breeding; thirdly, because nuclear instability is thought to have played a major role in crop plant evolution – understanding the past may help in predicting which new genome combinations will be successful crop species; fourthly, because failure to achieve adequate nuclear stability has played a major role in preventing so many potentially useful plants from becoming crops.

These points are illustrated mainly by reference to three different nuclear instabilities, namely: (1) haploid barley production by genome elimination in some *Hordeum vulgare* × *H. bulbosum* crosses; (2) the action of the *tri* gene in barley to produce about 50% diploid embryo sacs; (3) aberrant endosperm development in hexaploid triticale. Improved seed type in triticale has been achieved by a controlled reduction in rye telomeric heterochromatin. This approach may open the way for a new type of plant breeding, selecting for nucleotypic variation in the amount of non-coding DNA sequences.

Understanding the cellular mechanisms responsible for nuclear stability (or instability) is essential if controlled plant modification based on precise nuclear engineering is to become possible. This understanding can come only from sustained fundamental research.

1. Introduction

For the purpose of this paper, nuclear instability is defined as any deviant nuclear behaviour producing a nucleus (or nuclei) of abnormal structure, karyotype or behaviour, while plant breeding is interpreted in its widest sense to include cytogenetical research that has either already influenced crop variety production or might reasonably be expected to do so in the foreseeable future.

Shortage of space makes it impossible to review here the numerous types of nuclear instability that are known. Instead, this paper presents a few general comments about nuclear instability in the context of plant breeding and then mentions some of the important reasons why it merits sustained experimental attention in crop species and breeder's material. These points are illustrated mainly by reference to three examples of nuclear instability that I have recently studied, which occur at female meiosis or early in seed development. Thus, the present work is restricted to discussing aspects of nuclear instability in those temperate cereal grain crops (notably barley, bread wheat and hexaploid triticale) with which I am most familiar. It is hoped that the phenomena described, and the conclusions drawn, will prove to have a wider relevance.

2. Occurrence of nuclear instability in varieties

Higher plant species have been subject to prolonged natural selection for nuclear stability in their normal environments. Moreover, varieties of crops such as bread wheat and barley, bred for high fertility and uniformity, have also been subjected to intensive breeder's selection for nuclear stability. Nevertheless, nuclear instability occurs spontaneously in a typically very small proportion of cells of every individual, even in such crop varieties. For example, Riley & Kimber (1961) estimated the frequency of aneuploid progeny in four varieties of bread wheat (*Triticum aestivum*) and a variety of cultivated oat (*Avena sativa*) as 1.08% and 1.27%, respectively. These frequencies for hexaploids are typically much higher than in diploids. For example, Sandfaer (1973) estimated the frequency of aneuploid progeny in diploid barley (*Hordeum vulgare*) as only 0.003%. In general, the incidence of nuclear instability increases with the wideness of the cross. Aneuploidy is more frequent in crosses between than within bread wheat varieties.

An increased incidence of nuclear instability may be induced by many genetical and environmental factors including temperature (Bayliss & Riley 1972). Interestingly, nuclear instabilities may be much more frequent in diseased plants. Sandfaer (1973) showed that in 11 spring barley varieties infected with barley stripe mosaic virus, the incidence of aneuploid and triploid plants was respectively about 110 and 48 times higher than in healthy controls.

3. Some reasons why nuclear instability merits attention

Chromosomes and nuclei are self-replicating entities, so that a very low incidence of nuclear instability can have drastic consequences. Nuclear instability can occur in all cell types and throughout the life cycle. However, of greatest importance to the plant breeder are those instabilities that occur at significant frequencies either in the germ line affecting the phenotype of progeny, or in the endosperm and adversely affecting the expression of grain development. For example, aneuploidy (usually caused by pairing failure at meiosis) frequently results in reduced fertility and/or progeny with diverse phenotypes in wheat (Law & Worland 1973; Sears 1954) and barley (Tsuchiya 1967), while mitotic abnormalities in coenocytic endosperm are a frequent cause of sterility and misshapen grain (Moss 1970; Bennett 1974). Thus, nuclear instability merits attention first because it makes achieving the aims set by and for plant breeders (e.g. high fertility and uniformity) more difficult or impossible.

A second important practical reason for studies of nuclear instability concerns the legal and commercial standards of purity set for crop varieties. Riley & Kimber (1961) noted that the frequency of chromosomally variant types produced by nuclear instability in bread wheat is frequently greater than the incidence of morphologically distinguishable 'off-types' tolerated by some seed certification organizations. It is important that high standards of genetic purity are set and maintained in such inbred crops but, in that irregular chromosome constitutions may lead to phenotypic deviations (Sears 1954), it is essential that the required standards of genetic purity are biologically possible.

A general aim of the cytogeneticist is to understand the various causes and forms of nuclear instability, to be able to maximize nuclear stability within crops. In that other scientists are unlikely or unable to study chromosomes and nuclei, the cytologist has a unique responsibility

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to monitor their behaviour and misbehaviour, and to interpret his observations in relation to the plant breeder's aims and problems. In this connection it is important to remember that in wheat, barley, rye and many other cereals there is only a single female meiocyte, functional megaspore, egg and central cell per floret. In contrast with its occurrence on the male side of two-track heredity, the consequences of nuclear instability in these unique female cells are unavoidable. The basic unit of grain production is the floret and therefore the potential number of grains is the same as the number of fully formed florets. However, this potential is rarely if ever achieved in the field, the shortfall commonly ranging from only about 3–5% of first and second florets in the best crops of bread wheat varieties (J. Bingham, personal communication) to about 20% of florets in a two-rowed barley crop (Barling 1979). Nuclear instability in the female germ line has been recognized as one possible cause of sterility (Riley & Kimber 1961) but, as far as I am aware, its incidence has not been carefully quantified cytologically in any cereal grown either under equable conditions (when its importance is minimal) or under environmental stress such as drought, air-frost or high temperatures, when its practical significance may be greater. It is therefore suggested that such studies are long overdue because information on the incidence and causes of nuclear instability in the female germ line may indicate the need, if not the means, for a useful improvement in fertility in some crop plants.

4. Genome elimination

Although nuclear instability is generally undesirable because of its deleterious consequences, this is not invariably so. Indeed, there is one clear example in which spontaneously occurring nuclear instability has recently been manipulated to good effect by cytogeneticists; namely, genome elimination in certain hybrids.

Nuclear instability and chromosome loss is a common but variable phenomenon in interspecific hybrids, but in an extreme form all the chromosomes of one parent species are retained while all those of a second parent species are eliminated from dividing hybrid cells. Such genome elimination occurs in some crosses between diploid *Hordeum vulgare* and diploid *H. bulbosum*, where the *H. bulbosum* genome is eliminated leaving nuclei with a haploid *H. vulgare* complement (Kasha & Kao 1970; Subrahmanyam & Kasha 1973). Although elimination can occur at any time in the life cycle (Humphries 1978), it is frequently completed in young embryo cells soon after fertilization (Bennett *et al.* 1976).

Once the nature of the nuclear instability was understood, its advantage was soon realized. Thus, by using colchicine it is possible to double the chromosome number of haploid barley plants produced from a hybrid by genome elimination to give immediately homozygous plants (dihaploids), thereby removing the need for seven or eight generations of selfing from a barley breeding programme. This possibility has been applied in Cambridge and elsewhere in both spring and winter barley breeding programmes (although the time-saving over conventional breeding methods is greatest in winter barley) to produce thousands of dihaploids from plants in early generations. Two such lines produced at the Plant Breeding Institute, Cambridge, are showing promise and are being bulked before assessment in national trials, while in Canada a spring barley variety 'Mingo' produced by this means has been licensed for growing in Ontario only 5 years after the initial cross (Ho & Jones 1980).

Genome elimination in *Hordeum* is an example of nuclear instability that frustrated the

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purpose of the original interspecific cross (i.e. to make a hybrid) but was then applied in plant breeding after cytogeneticists had studied its nature, recognized its potential and developed its application. Knowledge of this interesting and useful phenomenon stems from a surprise result from wide-crossing experiments (Davis 1958). Indeed, neither its discovery nor its application were expected or planned. Thus, the recent history of this particular nuclear instability clearly shows how narrow the gap may be between fundamental cytogenetic studies and their practical application in plant breeding.

Similar genome elimination has been demonstrated in some hybrids between bread wheat and tetraploid *H. bulbosum* to yield wheat haploids (Barclay 1975). However, extending its application to wheat breeding has proved difficult because most varieties of bread wheat currently in agriculture possess genes that prevent crossing with *H. bulbosum* (Snape *et al.* 1979). Consequently, the possibility is being explored of producing dihaploids for use in wheat breeding programmes, either by ensuring that the genes for non-crossability with *H. bulbosum* are absent from future breeders' material, or by identifying clones of *H. bulbosum* less sensitive to their action (J. Snape, personal communication). Meanwhile, genome elimination is recognized as a potentially useful means of producing haploids in high frequencies in many other crops. Consequently, fundamental research is being conducted in several countries, to elucidate the cellular processes responsible.

5. Genome doubling in barley

Another example of nuclear instability in barley that seems to have potential for useful manipulation concerns genome doubling. Ahokas (1977) described a recessive mutant that he named triploid inducer (*tri*) in a single plant selection of the variety 'Paavo'. Diploid plants with 14 chromosomes homozygous for *tri* are fully viable and of normal fertility, but have two types of seed distributed at random within all spikes. One type has a normal endosperm and a diploid embryo, but the other has a thin endosperm and a triploid embryo. It was recently shown (Finch & Bennett 1979) that *tri* acts to produce chromosomally doubled functional megaspores (and subsequently unreduced egg nuclei) by suppressing the second meiotic division in about 50% of florets. However, *tri* does not similarly affect male meiosis.

Chromosome doubling with colchicine still proves difficult in some materials. It would be useful, therefore, if a gene like *tri*, which controls an exact doubling of the chromosome number, could be used to achieve doubling more easily, more frequently, or with a greater control of its timing. As a first step it was interesting to ask whether *tri* acts at higher ploidy levels. Autotetraploid *tri* plants were prepared by doubling a *tri* diploid with colchicine, and these produced about 50% hexaploid progeny, showing that *tri* acts at the higher ploidy level (R. A. Finch & M. D. Bennett, unpublished results). Hitherto, autohexaploid barley plants have been so rare as to merit special publication (Rommel 1960). By using *tri*, one hundred plants counted as hexaploids were easily obtained. Another potential use of *tri* is to make new genetic stocks more easily than before. For example, it should be possible to use the abundance of diploid and triploid *tri* progeny to make a new set of trisomics which, when crossed with *Hordeum bulbosum* should (after elimination of *H. bulbosum* chromosomes), yield significant numbers of tetrasomic plants with 16 chromosomes, if these are viable. Hitherto, such plants have been rare in barley (Tsuchiya 1967).

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6. Apomictic cereals

Unreduced gametes are known to occur at low frequencies (i.e. $< 1\%$) in many crop species, and are quite common on both the male and female side in a few sexual species (e.g. *Saccharum officinarum*), but they are most frequent in gametophytic apomicts. The notion of developing apomictic lines of cereal crops has certain attractions (Asker 1979), notably the possibility of fixing heterosis in crops such as hybrid maize or even bread wheat. Attention has mainly been focused on the possibility of introducing the gene complex controlling apomixis from another species by wide crossing. However, the way forward may lie in identifying within the crop species genes controlling the essential steps for apomixis, e.g. regular production of unreduced eggs, and parthenogenesis (the former by screening for high frequencies of triploids, and the latter by screening for high frequencies of haploids). In other words, it may be important to combine genes that fix as a regular feature of female germ line development something that, as an isolated event, would be termed nuclear instability.

7. Nuclear instability and evolution

The action of *tri* in causing genome doubling contrasts in evolutionary potential with that of the genes controlling genome elimination in many *Hordeum* species. If, as seems likely, both genes exist in wild *Hordeum* species then a prospect is opened of genomic 'snakes and ladders', with new polyploids being produced after genome doubling more easily and frequently than has been usually envisaged, and new diploids whose genomes have been modified during their association with other diploid genomes in polyploid species, being produced by genome elimination in hybrids. Genes in diploids that affect chromosome pairing in polyploids, but not apparently in the diploid itself (Dover & Riley 1972), may represent preadaptation for polyploidy, but they may also provide evidence of diploids being derived from polyploids by genome elimination.

Another reason why nuclear instability merits attention concerns the important role that it is believed to have played in crop plant evolution. For example, tetraploid and hexaploid wheats are thought to have arisen from parent diploids by hybridization followed by 'nuclear doubling', i.e. some unknown nuclear instability. Greater understanding of the evolutionary biology of crop genera should indicate the conditions for, and the means of, further or alternative crop improvement. Knowledge of the genetic architecture of bread wheat certainly contributed to the discovery of the *Ph* locus on chromosome 5B of bread wheat, whose activity is critical for maintaining nuclear stability at meiosis. The exciting and extensive manipulation of the wheat genome that this important discovery made possible is described in the accompanying papers by Thomas and Riley *et al.* (both in this symposium).

Realization of the important role of polyploidy in crop evolution resulted in the synthesis of a large number of new polyploids (see, for example, Bell & Sachs 1953), but few have become crops mainly because of nuclear instability at meiosis or in young seeds. For example, there were high hopes for autotetraploid barley (Nilan 1964). Initially its meiotic stability and fertility were poor but both were considerably improved by selection. However, the mechanism(s) responsible for the improvement are unknown. The pragmatic approach failed, and the improvement was just insufficient for its acceptance as a significant crop. Nevertheless, further understanding of the mechanisms of chromosome pairing in stable tetraploid species

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may suddenly provide a more rational basis for completing the development of many plants like autotetraploid barley as useful crops.

Attempts to construct new allopolyploid cereals have also been largely unsuccessful with the exception of hexaploid triticale, which seems certain to become a significant cereal crop species (Muntzing 1979). Triticale's incipient success shows that the concept of man-made crop species is feasible. However, triticale's development has been pragmatic, and consequently adds nothing to our ability to predict which other novel combinations of genomes can be made stable and productive in agriculture. This ability requires an understanding of the basic mechanisms determining nuclear stability or instability. Thus, an important value of triticale, whose whole development during about the past 30 years is known and open to investigation, is as a test-bed for experiments to establish the basis of its improvement. As an example of this approach, work on the cause and control of nuclear instability in triticale endosperm will be described.

8. Nuclear instability in triticale endosperm

In about 1970 it was clear that development of hexaploid triticale (comprising the chromosome complements of tetraploid *Triticum durum* and diploid *Secale cereale*) faced two major problems, namely sterility and shrivelled grain at maturity. Nuclear instabilities occur infrequently in young endosperms of all cereals, but observations of hexaploid triticale at this stage revealed a particularly extreme and frequent sequence of aberrant nuclear behaviour (Bennett, 1974). In wheat, rye and triticale, early endosperm development is coenocytic until about 1000–2000 nuclei are formed. Moreover, nuclear development within the coenocyte is highly synchronous, and the nuclear doubling time is very short compared with embryo and other cell cycle times (Bennett *et al.* 1975).

Nuclear instability in triticale endosperm often began with the formation of a chromatin bridge at anaphase (Bennett 1974). This would have been broken by cell wall formation in a cellular tissue but it persisted throughout interphase in the endosperm coenocyte. As a result, restitution nuclei were formed whose DNA contents doubled at successive rounds of DNA synthesis in the coenocyte. If the initial bridge occurred early in the coenocytic phase, then monstrous aberrant endosperm nuclei with 128, 256 or 512 times their normal DNA contents resulted, and the endosperm invariably aborted without becoming cellular. However, if the initial bridge formed late during the coenocytic phase, then cells with considerably higher ploidy levels than normal (but lower than those just mentioned) were formed at endosperm cellularization. Such aberrant cells, which were often formed in groups, subsequently either aborted or failed to contribute normally to endosperm cell division. Overcoming this instability would clearly depend on understanding the cause of the initial anaphase bridge.

Rye has about 35% more DNA than the largest diploid genome in tetraploid wheat. Moreover, all seven replicated rye chromosomes contain more DNA (4.4–4.9 pg) than the largest wheat chromosome (3.95 pg) (Gustafson & Bennett 1976). Unlike wheat chromosomes, rye chromosomes have large telomeric segments of heterochromatin on one or both arms that are selectively stained by Giemsa (Gill & Kimber 1974; Bennett *et al.* 1977). These segments account for about 18% of the total rye DNA (Bedbrook *et al.* 1980), are of recent origin, and contain some families of very highly repeated DNA sequences which are not detected in wheat (see also Flavell, this symposium). This and the fact that development is unimpaired or even improved after their deletion suggests that they are non-coding DNA. Lima-de-Faria & Jaworska

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(1972) showed that at DNA synthesis phase(s) in rye the telomeric segments are the last to replicate, and that the last 33% of S-phase involves synthesis of telomeric segments alone.

The significance of these interspecific differences in DNA amount and type is that both an increased DNA amount per diploid genome and an increased amount of late-replicating heterochromatin generally result in slower cell development. Moreover, rye and wheat are known to conform to this expectation. Thus, when rye and wheat are compared under controlled conditions, rye has a longer meiosis, pollen development time, cell cycle time in embryo

TABLE 1. THE PERCENTAGE OF ABERRANT NUCLEI IN 3 DAYS OLD COENOCYTIC ENDOSPERMS OF THE SEVEN DISOMIC ADDITION LINES OF HOLDFAST–KING II WITH AND WITHOUT DELETIONS OF TELOMERIC HETEROCHROMATIN

(From Bennett (1977).)

Addition	With deletion	Without deletion
I (5R)	—	0.51
II (6R)	—	1.30
III (2R)	0.28	—
IV (4R/7R)	—	2.75
V (1R)	—	1.05
VI (3R)	0.13	—
VII (7R/4R)	0.20	—
	mean 0.20	1.40

cells, nuclear doubling time in coenocytic endosperm, and S-phase in root-tip cells. Graves (1972) has shown that in hamster–mouse somatic cell hybrids, DNA synthesis begins synchronously in both complements, but the different durations and intragenomic patterns of synthesis in the two parent genomes are retained and appear to be regulated autonomously. If a similar situation applied in triticale, and the replication of the rye genome takes longer to complete S than wheat, then under certain circumstances rye chromosomes may enter mitosis (or meiosis) having failed to complete replication at one or both telomeres, resulting in bridge formation at anaphase.

The above observations and facts led to the development of a model, first published in 1974, for a causal chain linking rye heterochromatin with shrivelled grain in triticale. Thus, it was proposed that (1) late-replicating DNA (mainly telomeric heterochromatin) in rye chromosomes causes bridge formation at anaphase; (2) such bridges cause the production of abnormally polyploid endosperm nuclei; (3) that such aberrant nuclei cause either sterility (if they exceed a certain DNA *C*-value) or shrivelled grain (if they occur in significant numbers with lower DNA *C*-values). Thus, bridge formation causes either sterility or shrivelled grain depending on its timing during the coenocytic endosperm deveopment (Bennett 1974).

Evidence strongly supporting each step in this causal sequence has been obtained (Bennett 1977). First, C-banding of triticale endosperm mitoses has shown that anaphase bridges are invariably caused by rye chromosomes. Moreover, such bridges are usually caused by non-separation of telomers (the last-replicating segment). Secondly, further observations of triticale endosperm have repeatedly confirmed that persistent bridges develop into aberrant endosperm nuclei in disomic addition lines of King II rye to Holdfast wheat with or without deletions involving the loss of telomeric heterochromatin at one telomere. There was a significantly higher proportion ($p = < 0.05$) of aberrant nuclei in the four addition lines without the deletion

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of telomeric heterochromatin than in the three lines with such deletions (table 1). Thirdly, close positive correlations have been shown between the incidence of aberrant nuclei in coenocytic endosperm and the degree of shrivelling at maturity. The more aberrant nuclei there are during early endosperm development, the more shrivelled is the grain at maturity.

By 1976 the evidence that the presence of later-replicating DNA at rye telomeres was a major cause of grain shrivelling in many triticales was convincing. This provoked the question of what could be done to stabilize nuclear development at the critical stage of early endosperm development. It was decided to try to arrange for the absence of the rye telomeric heterochromatin in such plants. Four possible methods were considered.

1. To screen the genus *Secale* for taxa with less DNA and less telomeric heterochromatin than cultivated rye that could be used as parents for new primary triticales. Such taxa exist (Bennett *et al.* 1977).
2. To develop wheat–rye substitution lines. In about 1974 it became clear that most improved triticales from CIMMYT contained less than 14 rye chromosomes owing to their having wheat–rye substitutions (Merker 1975).
3. To utilize rye chromosomes occasionally found in triticale modified by the loss of some or all of the heterochromatin at one or both telomeres (Bennett 1977).
4. To develop a treatment that selectively removes telomeric segments from rye chromosomes in triticale.

In collaboration with J. P. Gustafson (University of Manitoba), I elected to explore the third possibility. It was therefore decided to screen hexaploid triticale lines for modified rye chromosomes with reduced or no heterochromatin at telomeres, to combine these in a common triticale background, and to study the effect on grain development.

TABLE 2. COMPARISON OF THE PERCENTAGE OF ABERRANT NUCLEI IN 20 ENDOSPERMS FIXED 72 h AFTER POLLINATION FOR PLANTS GROWN AT 20 °C, AND THE YIELD, TEST MASS AND 1000 KERNEL MASS IN FIELD-GROWN PLANTS FOR TRITICALE LINES DIFFERING FOR THE PRESENCE (+) OR ABSENCE (−) OF HETEROCHROMATIN ON THE SHORT ARM (S) OR THE LONG ARM (L) OF RYE CHROMOSOME 7, OR 6 AND 4.

(The number of location test years are given in parentheses.)

hexaploid triticale line	rye chromosome heterochromatin comparison	aberrant endosperm nuclei (%)	yield† t/ha	test mass† kg/hl	1000 kernel mass†/g
DR–IRA HH	7L+	5.2	4.79 (10)	65 (10)	45 (10)
DR–IRA EE	7L−	4.0	5.19*	64*	49**
1204	6S+ 4S+	6.8	2.47 (3)	62 (5)	48 (5)
1206	6S− 4S+	3.8	3.48**	63**	50**
1208	6S− 4S−	2.0	3.01	67	55

* $p = 5\%$; ** $p = 1\%$.
† Unpublished data, courtesy of Dr J. P. Gustafson, University of Manitoba.

Two rye chromosomes (4R and 6R) modified by the loss of heterochromatin in their short arms, were found by Gustafson in the line Miscellaneous 3636 and the variety Rosner, respectively. In addition, Merker (1976) described isogenic lines for the presence (HH) and absence (EE) of the largest telomeric C-band in the complement on rye chromosome 7 in the hexaploid triticale DR–IRA. Comparison of early endosperm development in lines homozygous for one

or two of these chromosomes with or without telomeric heterochromatin showed, as expected, that the proportion of aberrant endosperm nuclei at the coenocytic stage was reduced when rye telomeric heterochromatin was removed (table 2). Moreover, the effect was additive when two modified rye chromosomes were combined in line 1208 (figure 1). While these and similar results for other lines (M. D. Bennett & J. P. Gustafson, unpublished) are very encouraging, the crucial test of the value of the modified rye genome in triticale must be its performance in adequately replicated field trials. The first results from such tests (table 2) are also very encouraging. For example, both the yield and the mean kernel mass were about 8% higher in DR–IRA without heterochromatin on the long arm of 7R than in DR–IRA with such heterochromatin. Similarly, in line 1206 (with modified 6R) and 1208 (with modified 4R and 6R) mean kernel mass was respectively 4 and 14.5% higher than in line 1204 (with unmodified 4R and 6R). Moreover, test mass (which is often inversely proportional to shrivelling) showed a significant increase of 8% with reduced telomeric heterochromatin in these three lines.

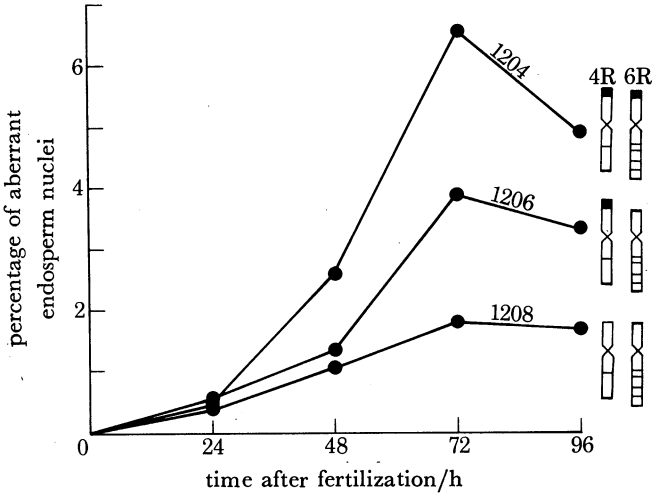

FIGURE 1. The mean percentage of aberrant nuclei in coenocytic endosperms fixed up to 96 h after pollination in triticale 1204, 1206 and 1208 lines with or without telomeric heterochromatin on the short arms of rye chromosomes 6 and 4.

Taken together, the available results seem to confirm that the presence of rye telomeric heterochromatin can have a major deleterious effect in many triticales on several interrelated grain characters of prime agronomic importance including shrivelling, kernel mass, fertility and yield. Consequently, work is continuing to produce triticales in which all of the rye chromosomes are modified by the loss of most, if not all, of their late-replicating heterochromatin. This work is funded in Cambridge by the Overseas Development Administration, and in Winnipeg by CIMMYT. Examples of five of the seven rye chromosomes, and seven of the eleven telomeres, have so far been identified in a modified condition. If they were all combined in a single line the normal appearance of the C-banded rye complement would be significantly altered, and would involve an estimated reduction in the DNA content of the rye genome in triticale of about 10%.

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9. A new dimension in plant breeding

It seems worthwhile to emphasize the novel features of the chromosome engineering being attempted in triticale. First, to improve its agronomic character it is intended to select for a controlled reduction in the mass of DNA in the rye genome in triticale of about 18%. There seems little doubt that this is feasible. Progress so far, involving three telomeres, has already involved an estimated reduction in the mass of rye DNA of about 4–5%. Secondly, most, if not all, of the DNA to be lost is non-genic, and the desired change does not involve selection for or changes in any coding sequences, but only for an improved nucleotype. Thirdly, if this approach results in an improved agronomic performance in triticale, a new dimension will have been added to plant breeding, since similar nucleotypic manipulation is potentially applicable to other interspecific hybrids whose parent species display nucleotypic differences similar to those between wheat and rye. Examples may include *Zea* × *Sorghum* and various interspecific pulse crop hybrids.

10. Conclusion

Studies of nuclear instability in germ line cells of crops and their wild relatives have been a source of interest and surprise, whose results have found practical application in plant breeding. It is reasonable to suppose that such speculative work will continue to provide new or improved means for achieving existing practical aims, and extending the range of nuclear manipulations at the plant breeder's disposal.

It is worth repeating that the success or otherwise of developing for agriculture so many apparently attractive polyploids and hybrids has turned on our ability to select lines with acceptably low rates of nuclear instability. This has been true for sexually derived material and no doubt it will yet determine the fate of the products of exciting new technologies, including new hybrids produced by protoplast fusion. When success has come in plant breeding, the reason why is often unknown as are the rational means of how to repeat it. Controlled plant modification, based on precise nuclear engineering, will come only from concerted and sustained fundamental research leading to a deeper understanding of basic cellular mechanisms, including those that determine nuclear stability and instabilities.

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