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Development of Kernel Shrivelling in Triticale

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With 4 figures and 4 tables

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Abstract

Shrivelling and poor sink performance of triticale kernels is caused by the replacement of sectors of the endosperm with internal cavities and by the poor growth of what normal endosperms remains. The cavities originate from patches of aberrant nuclei formed in the endosperm during the early coenocytic period by division errors. These errors mostly involve anaphase bridging of rye chromosomes. The role of delayed replication in the large terminal C bands of the rye chromosomes in including some or all of this bridging is controversial. However rapid coenocytic endosperm divisions, rapid growth in kernel dry weight in the early stages, atteinment of a large maximum kernel volume during development and high 1000 kernel weight at maturity all tend to aggravate kernel shrivelling in triticale.

Kernel shrivelling in triticale is not caused by a poor supply of substrates to the kernel nor by inefficient incorporation of these substrates into kernel dry matter. The premature increase in endosperm α-amylase activity which is observed in shrivelled triticale lines is closely associated with the microscopic endosperm and aleurone lesions. Although premature digestion of starch by this α-amylase undoubtedly contributes a little to the depression of dry matter accumulation in the kernels of shrivelled triticale lines it is probably a side effect of shrivelling rather than a major cause.

Triticale (× *Triticosesecale* Wittmack) refers to the hybrid complex derived from crossing between members of the wheat genus (*Triticum* L.) with the rye genus (*Secale* L.) (Gustafson 1976). It is believed to have potential as a cereal crop in its own right (Hulse and Spurgeon 1974, Zillinsky 1974, Zillinsky and Borlaug 1971). Triticale suffers from at least five reproductive disorders. These are meiotic irregularity, high rates of aneuploidy, partial

sterility, kernel shrivelling and premature sprouting of the embryo. Research on kernel shrivelling has advanced a lot in the last ten years. Some of the findings are reported in theses, symposia proceedings, research reports and other publications of limited availability. This paper seeks to evaluate this body of information and integrate it into a unified account of kernel shrivelling.

This review follows the time course of kernel development from fertilization of the female gametophyte onwards. It contrasts the normal pattern of development in the Triticinae with the abnormal development of triticale. In addition analogies are drawn between kernel shrivelling in triticale and the abnormal development of wheat-rye hybrid seed where these seem to be useful. To allow the reading of all sections by non-specialists we have also briefly reviewed background material where necessary.

Kernel Shrivelling in Triticale — Description and Quantification

In general, licensed varieties of common wheat and durum wheat produce plump kernels whereas rye may often produce slightly shrivelled grain samples (DARVEY 1973, KLASSEN 1970). Kernel shrivelling in unimproved triticales is usually severe and manifests itself in two ways. Firstly the endosperm may not grow fast enough to fill the pericarp (DRONZEK et al. 1974, KLASSEN 1970, ZILLINSKY 1973). Occasionally the endosperm may grow less on one side of the crease than on the other side (KLASSEN 1970). Secondly internal cavities are common within the endosperm itself (DEDIO et al. 1975, DRONZEK et al. 1974, KALTSIKES et al. 1975, KLASSEN 1970, SIMMONDS 1974, ZILLINSKY 1973). By the soft dough stage a large channel is usually found which runs along the crease between the vascular strand and the abaxial surface. Other cavities occur irregularly, both deep within the tissue of the endosperm and close to the surface where they can be seen before maturity as dark-looking patches under the pericarp. These abnormalities limit the capacity of the kernel to store and maintain as much carbohydrate and protein as possible. As the kernel dries out the complete or partial collapse of internal cavities gives the kernel an irregular outline. Even where the endosperm remains well formed the loose-fitting seed coat may be thrown into superficial folds.

Various indices can be used to quantify kernel shrivelling. Visual rating based on superficial appearance of the kernels works quite well. DARVEY (1973) rated shrivelling effects of individual rye chromosomes in wheat-rye addition lines as non-existent, mild, medium and severe. Other published scales have ranged from 1 to 10 or 1 to 13 with either low (KALTSIKES and ROUPAKIAS 1975, KALTSIKES et al. 1975) or high scores (BENNETT 1977) used to indicate better kernel types.

Three objective criteria have been used to quantify kernel shrivelling. These are test weight (FISCHER 1973), kernel density (KLASSEN et al. 1971, SALMINEN and HILL 1978) and a volume ratio (SALMINEN and HILL 1978). Test weight measures the density of the mature grain sample on a bulk volume

basis whereas kernel density measures the density on a displaced volume basis. The volume ratio is the displaced volume per kernel at maturity over the maximum volume which a kernel displaces during its development.

Kernel density of dry, non-shrivelled kernels of hard wheat is about 1.36 $g \cdot cc^{-1}$ (KLASSEN et al. 1971). However kernel density in triticale is often substantially less than this. KLASSEN et al. (1971) reported a range of densities for triticale between 1.30 and 1.08 $g \cdot cc^{-1}$ and for rye a density of 1.23 $g \cdot cc^{-1}$. SALMINEN and HILL (1978) reported a range of densities for triticale between 1.08 and 0.66 $g \cdot cc^{-1}$.

Test weight is the most easily measured of the three variables and it does correlate well with the large range of kernel densities observed in triticale (KLASSEN 1970 reported an r value of 0.933**). However test weight can also be influenced by packing properties of the grain unrelated to shrivelling, especially kernel shape (HLYNKA and BUSHUK 1959).

The volume ratio is probably the most laborious variable to determine. It measures the extent of external shrinkage as the kernel matures and dries out. In triticale this includes the partial or complete collapse of internal cavities. On the other hand mature kernel density measures the extent of cavities which remain within the kernel after it has dried out.

SALMINEN and HILL (1978) found no significant correlation between the volume ratio and kernel density. This suggests that the extent to which kernel abnormalities during development translate at maturity into either volume reduction or internal cavities varies from one line of triticale to the next. Consequently none of these variables is an ideal index of shrivelling. Perhaps the overall degree of shrivelling might best be measured by a joint index such as dry weight at maturity over maximum volume displacement during development.

The Embryo Sac and Fertilization

In the Triticinae a mature, unfertilized embryo sac consists of a large, pear-shaped vesicle that contains 2 synergids, 1 egg cell, 2 polar nuclei and several antipodal cells (BENNETT et al. 1973, 1975, CASS and JENSEN 1970, MORRISON 1955, POPE 1937). The antipodals are located in the broad chalazal end of the embryo sac. They contain large polyploid nuclei with enormous nucleoli. At the time of fertilization these nuclei are in an interphase-like condition (KALTSIKES 1973). It has been suggested that the antipodals function as a secretory or glandular tissue for the nutrition of the growing embryo and endosperm after fertilization (BENNETT et al. 1975, MAHESHWARI 1950). In our view another likely role is the synthesis of the cytoplasm of the large central cell before fertilization (KALTSIKES et al. 1975).

The two polar nuclei are appressed to each other (BENNETT et al. 1975) and lie within a strand of cytoplasm that traverses the vacuole of the central cell from the narrow micropylar end to the broad chalazal end (CASS and JENSEN 1970, WOJCIECHOWSKA and LANGE 1977). The two pyriform synergids

lie on either side of the pyriform egg cell at the micropylar end of the embryo sac and point towards the chalazal end (BENNETT et al. 1975). The egg and polar nuclei are in a prophase-like condition and large whereas the synergids are in interphase and smaller.

Gross abnormalities in the egg sac of triticale such as 3 polar nuclei or replacement of the gametophyte by parenchyma are probably associated with sterility rather than with shrivelling (BENNETT 1973, BENNETT et al. 1975). In many hexaploid lines of triticale which shrivel, the majority of gametophytes are normal in appearance (BENNETT et al. 1975, KALTSIKES 1973, KALTSIKES et al. 1975). While aneuploid seed may show increased shrivelling (KLASSEN 1970), euploid seed can also shrivel badly. Therefore aneuploidy of the gametes cannot explain why triticales shrivel so characteristically.

Compared to wheat, both rye and triticale produce fewer antipodals (BENNETT et al. 1975) and both tend to shrivel (DARVEY 1973, KLASSEN et al. 1971). However, within hexaploid triticale itself, lines which shrivel badly at maturity tend to produce a relatively high number of antipodals per egg sac. The pooled correlation of shrivelling rank (least shrivelled was ranked first) and the number of antipodals was calculated from data of BENNETT (1977), BENNETT et al. (1975) and KALTSIKES (1973) and was found to be significant ($r = +0.688$, d.f. > 7.0, $p < 0.05$). Consequently, no quantitative or qualitative differences in ovule morphology have been described which might indicate why triticales are predisposed to kernel shrivelling.

After pollen arrives on the stigma but before a pollen tube reaches the micropyle, one synergid begins to degenerate (CASS and JENSEN 1970). A single pollen tube reaches the micropyle in 40 minutes or less after pollination (BENNETT et al. 1973, 1975, WOJCIECHOWSKA and LANGE 1977). It enters the female gametophyte through the wall of the degenerate synergid bordering the micropyle (CASS and JENSEN 1970). One sperm nucleus is released into the central cell where it migrates along the axial cytoplasmic strand to reach and fuse with the polar nuclei (CASS and JENSEN 1970, WOJCIECHOWSKA and LANGE 1977). First mitosis of the resulting primary endosperm nucleus occurs around 6 to 8 hours after pollination (BENNETT et al. 1973, 1975, KALTSIKES 1973, POPE 1937, WOJCIECHOWSKA and LANGE 1977). The other sperm nucleus enters the egg cell, probably from the chalazal end of the degenerate synergid (CASS and JENSEN 1970) and becomes appressed to the egg nucleus (WOJCIECHOWSKA and LANGE 1977). First mitosis of the zygote occurs 22 to 36 hours after pollination (BENNETT et al. 1973, 1975, KALTSIKES 1973, MORRISON 1955, POPE 1937, WOJCIECHOWSKA and LANGE 1977). Variations in the rate of development among different species and varieties appear minor (BENNETT et al. 1973, 1975, WOJCIECHOWSKA and LANGE 1977). Rye pollen takes a little longer to fertilize wheat than does wheat pollen (WOJCIECHOWSKA and LANGE 1977, MEYER 1971). No variations in fertilization were reported by either BENNETT et al. (1975) or KALTSIKES (1973) which related to seed shrivelling in triticale.

When the pollination of common wheat with rye was delayed with respect to the physiological age of the wheat ovules there was increased

shrivelling of the resulting wheat × rye hybrid seed (THOMAS and ANDERSON 1978). Similar shrivelling was also observed following delayed pollination in intervarietal wheat × wheat crosses but to a much lesser extent. It would be interesting to know if there is genetic variation in the timing of anthesis relative to the age of the ovule and if this could increase or decrease the predisposition of triticale to produce shrivelled kernels. The influence of ovule age on the appearance of the embryo sac and on the cytological details of kernel development also needs to be studied.

The Coenocytic Phase of Kernel Development

After its first mitosis the endosperm enters a period of coenocytic growth with synchronous or near synchronous cycles of nuclear division. The time required for the nuclear cycle is initially very short (about 5 to 6 hours) but after 48 hours it gets progressively longer (BENNETT et al. 1973, KALTSIKES 1973, KALTSIKES et al. 1975). After fertilization the volume of the ovule begins to grow (KALTSIKES 1973) but the endosperm nuclei divide even faster so that the cytoplasm of the central cell becomes filled with nuclei. As the endosperm nuclei grow in number they begin to show gradients in their nuclear stage. In the early divisions these gradients are absent or slightly precocious toward the embryo but later on the asynchrony becomes greater with precocious nuclear stages toward the chalazal end and retarded stages around the embryo (BENNETT et al. 1975, KALTSIKES 1973). Since these later gradients persist until cellularization it is concluded that all endosperm nuclei are mitotic until the end of the coenocytic period (BENNETT et al. 1975, KALTSIKES et al. 1975).

Compared to the endosperm, division in the embryo is slow and cellular (BENNETT et al. 1973, 1975, KALTSIKES 1973, MORRISON 1955, WOJCIECHOWSKA and LANGE 1977). For instance, 72 hours after pollination the 22 genotypes studied by BENNETT et al. (1975) contained an average of 18.7 cells per embryo compared to 1350 nuclei per endosperm. Increases in polyploidy may increase the rate at which the embryo divides and grows although genetic effects among varieties of the same species may also be considerable (BENNETT et al. 1975).

After fertilization the antipodal nuclei begin to change. They pass through a prophase-like and then a metaphase-like condition during which the polynemic character of the chromosomes is visible before they degenerate (KALTSIKES et al. 1975). Degeneration of the antipodals roughly coincides with the end of the coenocytic period and the beginning of cellularization (KALTSIKES and ROUPAKIAS 1975, KALTSIKES et al. 1975). Cellularization usually begins in the neck area of the endosperm, close to the embryo about 72 hours after pollination and about the time of the 10th nuclear doubling (BENNETT et al. 1975, KALTSIKES and ROUPAKIAS 1975, KALTSIKES et al. 1975, SHEALY and SIMMONDS 1973, SIMMONDS 1974) and is completed 24 hours later (BENNETT et al. 1975). However variations occur.

The first clear sign of abnormality in the kernel development of triticale is the appearance of abnormal-looking nuclei in the coenocytic endosperm (BENNETT 1973, 1977, KALTSIKES et al. 1975). These aberrants often occur in patches. Both the total number and the proportion of aberrant nuclei increases during the coenocytic period. In comparison the coenocytic period of endosperm division in wheat is relatively free of abnormality (KALTSIKES et al. 1975, MORRISON 1955).

The endosperm aberrants can be subdivided into a number of different categories based on their shape, ploidy, stage of division and appearance (KALTSIKES et al. 1975, *Table 1*). At mitosis the most common type of abnormality in the endosperm is the anaphase bridge. BENNETT (1973, 1977) proposed that most aberrant cell lineages begin with the formation of an anaphase bridge. A scheme based on this idea is presented in *Figure 1*. It shows how the different kinds of aberrants listed and illustrated by KALTSIKES et al. (1975) could be derived from the various consequences of anaphase bridging. Since there is no cell wall formation, anaphase bridging would frequently lead to restitution and the formation of dumbell and polyploid nuclei.

The frequency of aberrants in the coenocytic endosperm shows a high positive correlation with the degree of kernel shrivelling at maturity both in triticale and in wheat-rye additions (BENNETT 1977, KALTSIKES and ROUPAKIAS 1975, KALTSIKES et al. 1975). Wheat-rye additions are lines in which the complement of wheat chromosomes has been supplemented by a single pair of homologous rye chromosomes. These high correlations between the frequency of endosperm aberrants and the degree of kernel shrivelling suggest a causal link between the two. However the actual number of aberrants is often not that great compared to the remaining total of normal-looking nuclei (KALTSIKES et al. 1975). Therefore the aberrants must have an influence out of proportion to their numbers.

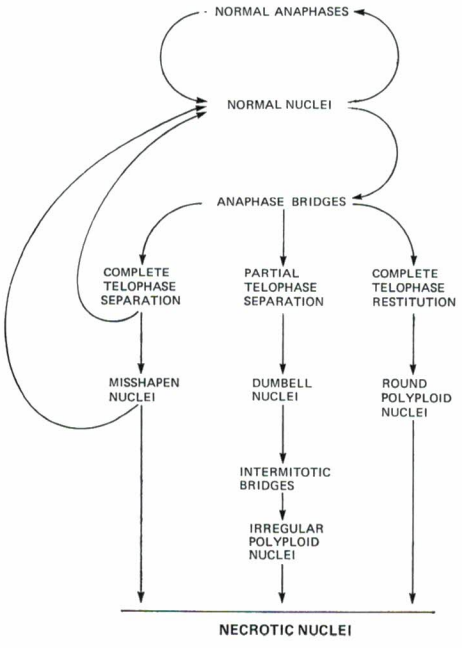

Fig. 1 Suggested interrelationships among normal and abnormal coenocytic endosperm nuclei in triticale: on this scheme all abnormalities described by KALTSIKES et al. (1975) can be initiated by an anaphase bridge

Tab. 1 Correlations of seed shrivelling score with the frequency of various classes of aberrant endosperm nuclei among triticales and among wheat-rye addition lines

Type of aberrant nucleus	Triticales*)		Wheat-rye addition lines**)	
	average frequency per 1000 nuclei	correlation with seed shrivelling score	average frequency per 1000 nuclei	correlation with seed shrivelling score
Total	12.78	+0.978 **	9.63	+0.872 ***
Polyploid — Total	7.56	+0.963 **	4.94	+0.853 **
— Round	0.05	+0.688 ns	2.54	+0.797 **
— Irregular	7.51	+0.964 **	2.40	+0.683 *
Misshapen	3.28	+0.237 ns	1.36	+0.671 *
Necrotic	0.80	+0.841 ns	2.09	+0.752 *
Dumbell	0.34	+0.850 ns	0.09	+0.429 ns
Bridges	0.28	+0.818 ns	0.41	+0.544 ns
No. of lines studied	4		10	

*) Calculated from data of KALTSIKES et al. (1975).

**) Calculated from data of KALTSIKES and ROUPAKIAS (1975). Addition lines 4RL and 6RS were omitted because of small sample sizes.

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ns: non-significant.

Among the aberrants the polyploid nuclei appear to be the most important category based on three criteria. Firstly, compared to other classes of aberrant nuclei there are high correlations between their individual frequency and the associated degree of shrivelling (*Table 1*). Secondly, polyploid nuclei are the most frequent class of aberrant (KALTSIKES and ROUPAKIAS 1975, KALTSIKES et al. 1975). Thirdly, because they are polyploid they contain a disproportionately large amount of the total nuclear material. Once formed these polyploid nuclei may continue to undergo rounds of nuclear duplication without division for some time, in synchrony with the normal, dividing nuclei (BENNETT 1973, 1977). Consequently the partition of nuclear material between the normal and the abnormal nuclear populations might be a better measure of the total degree of aberrancy than the mere frequency of abnormal nuclei. In extreme cases division failure of the primary endosperm nucleus may produce an endosperm that contains only one gigantic polyploid nucleus so that no normal nuclei are formed at all (BENNETT 1973, KALTSIKES and ROUPAKIAS 1975). Even when such a disaster does not occur the average loss to the normal nuclear population from the formation of aberrant nuclei may be considerable. Calculations for the shrivelled triticale 6A190 suggest that the formation of irregular polyploid nuclei alone reduces the potential size of the nuclear population by about 1/5th (KALTSIKES et al. 1975).

Shrivelling in triticale can be attributed to the rye component rather than the wheat component. For one thing compared to wheat, rye itself forms more aberrant nuclei within the endosperm during the coenocytic period (KALTSIKES et al. 1975, Moss 1970) and also tends to produce shrivelled grain at maturity (DARVEY 1973, KLASSEN 1970). Secondly, considering triticales as a whole, the degree of kernel shrivelling is more severe than it is in comparable amphidiploids between wheat and other diploids such as wheat-*Aegilops* or wheat-*Agropyron* (personal observation). Thirdly, with the possible exception of chromosome 2R, all the chromosomes of rye, when added individually to wheat have shrivelling effects on the kernel which range from mild to severe (DARVEY 1973). Fourthly the effects of these additions of rye chromosomes to wheat correlate positively with their effects on the frequency of aberrant endosperm nuclei (KALTSIKES and ROUPAKIAS 1975, *Table 1*). Finally karyotypic analysis of bridged chromosomes in the endosperm has shown that these are nearly always rye chromosomes (BENNETT 1977). This positive identification of rye chromosomes was achieved with C-band techniques (GILL and KIMBER 1974 a, SARMA and NATARAJAN 1973, VERMA and REES 1974). Large terminal C-bands are found at up to 11 out of the 14 telomeres of the rye genome (DARVEY and GUSTAFSON 1975, MERKER 1975, VOSA 1974). Although wheat chromosomes lack the large terminal bands which are characteristic of the rye chromosomes, wheat chromosomes frequently show small terminal bands and both groups carry small intercalary bands (DARVEY and GUSTAFSON 1975, GILL and KIMBER 1974 a, 1974 b, GUSTAFSON and KROLOW 1978). The large terminal bands of the rye chromosomes probably contain DNA that is repetitive and that is late replicating relative to median and proximal euchromatin, to chromatin at the centromeres and to the chromatin of B chromosomes (APPELS et al. 1978, AYONOADU and REES 1973, DARLINGTON and HAQUE 1966, DEUMLING 1978, LIMA-DE-FARIA and JAWORSKA 1972). There is some evidence of sequence homologies within the rye chromosomes, between the large, late replicating terminal bands and certain intercalary sequences (APPELS et al. 1978, R. B. FLAVELL, personal communication). Whether these intercalary sequences correspond to intercalary C-bands or whether any of the small, intercalary bands of wheat or rye chromosome are late replicating is unknown.

BENNETT (1973, 1977) suggested that anaphase bridging of rye chromosomes occurred in the endosperm of triticale when endosperm nuclei divided before DNA replication of the large, late replicating terminal C-bands was completed. As evidence for this he compared the C-band pattern of the 7 rye additions to hexaploid wheat (Holdfast-King II series) with their effects on the percentage of aberrant nuclei in the coenocytic endosperm. Chromosomes 2R, 3R and 7R present in these additions have each been modified by a deletion which has eliminated a major C-band from one arm compared to their karyotype in rye and each produces relatively few aberrants (BENNETT 1977, SINGH and RÖBBELEN 1976). In comparison, chromosomes 1R, 4R, 5R and 6R all produce conspicuously greater numbers of aberrants in their respective wheat-rye additions and their karyotypes remain intact.

On the other hand the effects of the telocentrics of chromosomes 4R, 5R and 6R in wheat-rye additions do not support a direct relationship between the large C-bands on the one hand and aberrant nuclei and kernel shrivelling on the other. Chromosomes 4R, 5R and 6R have particularly severe shrivelling effects on the kernels of wheat-rye addition lines (KALTSIKES and ROUPAKIAS 1975, DARVEY 1973). However in each case the effects of the whole chromosome on the frequency of aberrant nuclei and on kernel shrivelling is associated with the long arm which does not carry a major terminal C-band (*Table 2*, note that data for aberrants in $5R^L$ is lacking). By contrast the short arm of each of the added chromosomes does carry a major terminal C-band but in each case the short arm is without any marked effect on either aberrant frequency or kernel shrivelling (*Table 2*).

Tab. 2 Effect of long and short arms of rye chromosomes 4R, 5R and 6R on seed shrivelling and aberrant frequency in wheat-rye addition lines in relation to the location of large terminal heterochromatin bands

Chromosome		Presence of heterochromatin	Shrivelling		Aberrants per 1000 nuclei	
			ND*)	K&R**)	MDB***)	K&R**)
4R	Whole chromosome	+	yes	6.5	27.5	9.6
	Long arm only	—	yes	4.5	—	8.0
	Short arm only	+	no	1.0	—	0.5
5R	Whole chromosome	+	yes	8.5	5.1	19.5
	Long arm only	—	yes	—	—	—
	Short arm only	+	no	1.0	—	1.6
6R	Whole chromosome	+	yes	7.5	13.0	29.3
	Long arm only	—	yes	4.5	—	10.1
	Short arm only	+	no	1.0	—	1.9

*) Data of DARVEY (1973) based on Chinese Spring-Imperial, Holdfast-King II and Kharkov-Dakold series.

**) Data of KALTSIKES and ROUPAKIAS (1975) based on Holdfast-King II series except for 5R whole chromosome which is Kharkov-Dakold.

***) Data of BENNETT (1977) based on Holdfast-King II series exclusively.

In triticale the evidence for a direct relationship between C-bands and kernel shrivelling is also contradictory. For instance, although the bridged chromosomes observed within the coenocytic endosperm of triticale generally are rye chromosomes, they occur in chromosome arms which lack major terminal C-bands as well as in chromosome arms which have the large, terminal bands (BENNETT 1977). Accumulation of up to three rye chromosomes which had been modified by the deletion of a heterochromatin band in single triticale lines progressively reduced the number of aberrant nuclei present in the coenocytic endosperm. Unfortunately since these comparisons cannot yet be

made in an isogenic background they are confounded by genetic differences. On the other hand, isogenic comparisons for two of these chromosomes suggest that singly, the losses of the heterochromatic band form the long arm of 7R and from the short arm of 6R have no material on the degree of kernel shrivelling in triticale (A. MERKER, personal communication, P. J. KALTSIKES, personal observation). More generally, whereas 6A250 produces better seed than 6A190 and has less heterochromatin (J. P. GUSTAFSON, personal communication) the triticale Beagle tends to produce better seed than the triticale DRIRA although of this pair it has the more heterochromatin of the two (A. MERKER, personal communication).

All these contradictions could be resolved if it were found that the small intercalary bands of rye chromosomes were also late replicating and involved in internuclear bridging in the endosperm (J. P. GUSTAFSON, personal communication). However, against this possibility is the observation that the ". . . bridges are invariably caused by failure to separate chromatids at the telomere . . ." (BENNETT 1977). Consequently the exact cytological features of rye chromosomes which are responsible for bridging and the production of aberrant nuclei remains unclear.

If bridging in the endosperm of triticale were caused by division of the nucleus before the rye chromosomes finished replicating their heterochromatin then one might expect an association between rapid rates of endosperm division and increased production of aberrant nuclei. Rapid endosperm division would tend to magnify any differences in rate or time of replication between wheat and rye chromosomes. Comparison of hybrid seed of common wheat crossed with rye with the seed of common wheat itself shows that the substitution of the male wheat gamete by a rye gamete in the endosperm leads

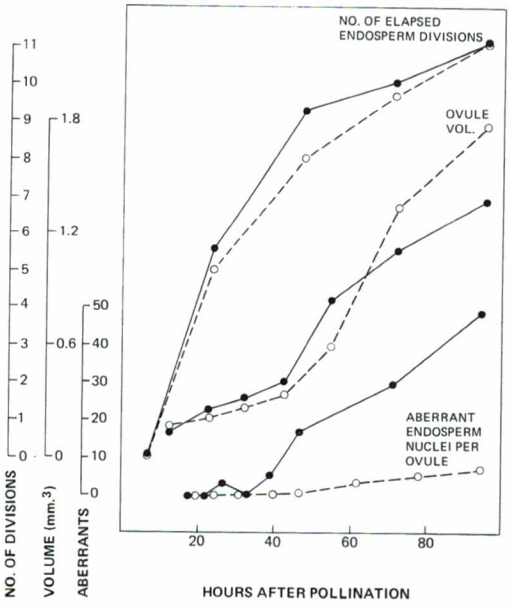

Fig. 2 Changes in the number of elapsed nuclear cycles, ovule volume and aberrant endosperm nuclei during the coenocytic period of endosperm growth in a shrivelled and a non-shrivelled triticale amphiploid. Data of KALTSIKES (1973) and KALTSIKES et al. (1975). Closed circles and solid lines are 6A190 (shrivelled) and open circles and dotted lines are 6A250 (non-shrivelled)

to faster rates of nuclear division, increased production of aberrant nuclei and obvious shrivelling at maturity (Moss 1970, Thomas and Anderson 1978, Wojciechowska and Lange 1977). Compared to the plump seeded triticale 6A250, 6A190 shows faster rates of endosperm division in the early part of the coenocytic period, produces greater numbers of aberrant nuclei later on in the coenocytic period and forms larger kernels that shrivel badly at maturity (*Fig. 2*, see also Kaltsikes 1973, Kaltsikes et al. 1975, Klassen et al. 1971, Saari 1977, Salminen and Hill 1978). Both 6A190 and 6A250 are raw amphiploids derived directly from crosses between tetraploid wheat and rye.

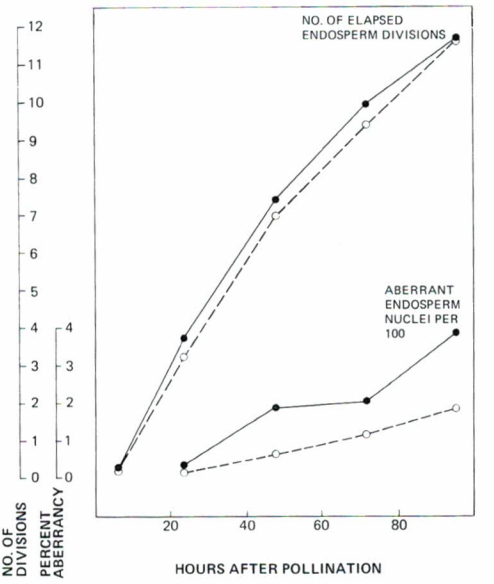

Fig. 3 Changes in the number of elapsed nuclear cycles and aberrant endosperm nuclei during the coenocytic period of endosperm growth in shrivelled and non-shrivelled advanced lines of triticale. Data of Bennett (1977) and M. D. Bennett (unpublished). Closed circles and solid lines are means of the three most shrivelled lines and open circles and dotted lines are means of the three least shrivelled lines

A similar association between fast division rates in the endosperm, increased production of aberrant nuclei and increased shrivelling at maturity seems to exist among secondary hexaploid triticales (*Fig. 3*, Kaltsikes et al. 1975). These secondary hexaploids are the products of intercrossing triticales and selection. Their third ("rye") genome is often substantially modified by a reduction in chromosome size. This occurs either by the deletion of heterochromatin or by the substitution of rye chromosomes with their corresponding homoeologues from the D genome of common wheat (Gustafson and Bennett 1976, Darvey and Gustafson 1975, Gustafson and Zillinsky 1978, Merker 1975). These changes in the secondary types seem to have been accompanied by a reduction in the rate of endosperm division compared to the parental species of wheat and rye (Bennett et al. 1975) and also compared to raw amphiploids (Kaltsikes 1973, Kaltsikes et al. 1975). Part of these changes could be the result of selection by plant breeders for improved seed type in segregating populations of triticale (Bennett 1977, Gustafson and Bennett 1976).

Fast rates of endosperm division in the coenocytic period also seem to correlate positively with the number of antipodal nuclei present in the embryo sac. Correlation between the number of elapsed endosperm divisions 72 hours after pollination and the number of antipodals per embryo sac for the 22 wheats, barleys, triticales et cetera studied by BENNETT et al. (1975) was significant ($r = +0.642$, d.f. = 20, $p < 0.01$). Since the antipodals and the endosperm are closely related ontogenetically, a tendency toward rapid endosperm division could also increase the number of divisions among the antipodals while they are still mitotic. An association in triticale between high numbers of antipodals on the one hand and rapid endosperm division and kernel shriveling on the other would explain the correlation between the number of antipodals and kernel shrivelling which was noted earlier in this paper.

These findings support the notion of a causal link between fast rates of nuclear division in the coenocytic endosperm, increased frequencies of aberrant nuclei arising from these divisions and increased shrivelling at maturity. Against this there are two points. The first inconsistency is that none of the five advanced lines studied by KALTSIKES and coworkers produced better seed than 6A250 despite having slower rates of endosperm division (KALTSIKES 1973, KALTSIKES et al. 1975). However, since on average kernel shrivelling is increased in triticales of short plant height (personal communication from CIMMYT triticale breeders R. RODRIGUEZ, F. J. ZILLINSKY and B. SKOVMAND) it might be better to avoid direct comparison of old, tall amphidiploids like 6A250 with the shorter products of inter-triticale breeding and selection.

The second inconsistency is that while the rate of division declines continuously right through the coenocytic period, aberrants do not begin to appear in any great numbers until over half the division cycles are completed (BENNETT 1977, BENNETT et al. 1973, 1975, KALTSIKES 1973, KALTSIKES and ROUPAKIAS 1975, KALTSIKES et al. 1975) and the percentage of aberrancy may actually increase right up until the last and slowest coenocytic divisions (*Fig. 3*). The behaviour of the shrivelled line 6A190 is particularly interesting. Compared to 6A250, the endosperm of 6A190 seems to enter a period of stress about halfway through the coenocytic period. About 48 hours after pollination 6A190 loses its early advantage in division rate, slows down its rate of growth in volume and begins to produce aberrant nuclei in large numbers (*Fig. 2*). Therefore the bridging of rye chromosomes in the endosperm cannot be a direct consequence of rapid divisions. Instead it may be triggered by stress conditions which are themselves created by a prior period of rapid division; the faster the prior divisions then the greater will be the ensuing stress. Maybe the rapid coenocytic growth causes the endosperm to run short of DNA precursors, slowing down DNA synthesis and causing bridging where mitosis overtakes replication (M. D. BENNETT, personal communication). Several features of the coenocytic endosperm suggest that even under normal conditions demand for DNA precursors will be high. These are triploid endosperm (in triticale $3n = 9x$), synchronous divisions and short nuclear cycle times. When a polyploid is first synthesized the enlarged number of chromosomes will increase this demand for precursors. Rye chromosomes are com-

paratively big (GUSTAFSON and BENNETT 1976, KALTSIKES 1971), they carry large amounts of late-replicating DNA and have evolved under diploid and not polyploid conditions. Therefore added stress from the ploidy increase in triticale should fall most heavily on them. It is suggested that when unmodified rye chromosomes are placed in the larger and more demanding nuclei of triticale and they then attempt to replicate for prolonged periods under fast cycle times, they exhibit cytological limitations which lead to bridging and the formation of aberrant nuclei and this ultimately leads to shrivelling.

The Cellular Phase of Kernel Development

By 24 hours after cellularization all evidence of nuclear synchrony has disappeared (BENNETT et al. 1975). As the endosperm gets bigger there is a shift to peripheral mitoses and by 10 days after anthesis the outermost layer appears to be functioning as a meristem layer, which divides both radially and tangentially except around the pigment strand. This meristem is also the aleurone layer (EVERS 1970, SIMMONDS 1974). Failure to form a meristem around the pigment strand results in involution of the adaxial surface, so forming the crease. Starting from the aleurone layer, endosperm cells can be divided into three types (EVERS 1970), SIMMONDS 1974). These are small, isodiametric sub-aleurone cells, larger radially elongated prismatic cells and isodiametric central cells around the crease.

Small primary starch granules are present in the very young endosperm but these disappear by the middle of the coenocytic period. Secondary starch granules first appear about the time of the 8th nuclear doubling (KALTSIKES and ROUPAKIAS 1975, KALTSIKES et al. 1975) which is prior to the beginning of cellularization. Thereafter starch formation develops rapidly in the endosperm (DRONZEK et al. 1974, SIMMONDS 1974).

In the early stages the deposition of dry matter proceeds more slowly than the growth in kernel volume. In western Canada the wheat kernel reaches its maximum volume about 4 weeks after anthesis whereas grain filling takes about 6 to 7 weeks (personal observation). Grain filling appears to delay senescence since heads without kernels senesce more rapidly than those which carry seed (THOMAS and ANDERSON 1978). Toward the end of the grain filling period the kernels start to lose chlorophyll and dry out and the tillers which bear them senesce and die. By the time the moisture of wheat kernels reaches about 35 %, grain filling has ceased (DODDS 1957, DODDS et al. 1979).

In shrivelled triticales, cellularization of the endosperm and the sequence of changes which lead to antipodal degeneration seem to begin after slightly fewer divisions in the endosperm than is the case in plump seeded triticales which in turn may initiate these changes after fewer divisions than wheat (KALTSIKES et al. 1975). In rye, cellularization is delayed as in wheat but antipodal degeneration begins early as in triticale. Abnormalities are also present in the endosperm of triticale after cellularization as well as during the earlier coenocytic period. Cavities occur within the endosperm, both around the

margin and in central locations, especially around the bottom of the crease (DEDIO et al. 1975, DRONZEK et al. 1974, KALTSIKES et al. 1975, KLASSEN 1970, SIMMONDS 1974). These cavities contain no recognizable contents and do not deposit starch (DEDIO et al. 1975, SIMMONDS 1974). They probably originate from patches of aberrant nuclei formed during the coenocytic period since such nuclei are incapable of forming normal endosperm tissues. Best evidence of this inability comes from crosses between wheat and rye. In hybrid seed between tetraploid wheat and rye, nuclei of the coenocytic endosperm are completely converted to polyploid and other abnormal nuclei by division errors, notably anaphase bridges (MOSS 1970). The endosperm tissues found two to three weeks after pollination which derive from these nuclei usually fill only a small part of the interior of the seed, are watery and do not deposit starch so that the hybrid seed collapses at maturity (KALTSIKES 1974, MOSS 1970, TAIRA and LARTER 1977). In contrast the endosperm nuclei of hybrid seed between the common wheat variety 'Chinese Spring' and rye are only 10% abnormal and the majority of hybrid seed in this cross are viable, contain a large, starch-laden endosperm and show only partial shrivelling at maturity (MOSS 1970, THOMAS and ANDERSON 1978, WOJCIECHOWSKA and LANGE 1977). In short, endosperm breakdown in hybrid seed of tetraploid wheat and rye appears to be an extreme version of the kernel shrivelling which exists in triticale and in hybrid seed of hexaploid wheat and rye.

In addition to the cavities, meristematic activity of the remaining endosperm tissues may also be abnormal. Cavities themselves could affect the pattern of development in adjacent sectors where normal endosperm tissue is present. Cells adjacent to a cavity will be unsupported by tissue pressure on that side thereby altering their pattern of division and expansion. Planes of division in the peripheral meristem and the expansion of division products may be sufficiently irregular that the palisade appearance of the prismatic cells is not established (see SIMMONDS 1974: page 111, 114 and Figure 7b). Sections of the peripheral meristem (aleurone) may be missing or distorted (DEDIO et al. 1975, SIMMONDS 1974). Missing sections of aleurone are probably associated with adjacent endosperm cavities (SIMMONDS 1974). Involution of the aleurone could also be simulated if a sub-nucellar cavity were partially reclaimed by mitoses in the normal cells which border the cavity (SIMMONDS 1974: Figure 7d). In other cases the nucellar epidermis may hypertrophy and intrude into the aleurone and endosperm (SIMMONDS 1974).

Probably most of the developmental abnormalities of the cellular period derive from the misdivisions in the earlier, coenocytic period. The overall pattern of kernel growth also suggests that the degree of shrivelling at maturity is determined early on in the development of the kernel. If kernel dry weight is expressed per unit of the maximum volume which a kernel reaches during its development then dry matter accumulation in the shrivelled triticale lines is seen to be depressed right from the earliest stages (SALMINEN and HILL 1978). On the other hand if dry weight is expressed per kernel then the opposite is true. Low kernel density at maturity is associated with rapid increase in the dry weight of the whole kernel in the first ten days after anthesis (*Fig. 4,*

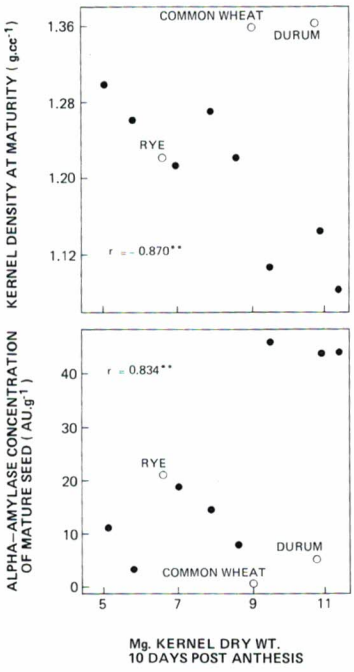

Fig. 4 Kernel density and α-amylase activity at maturity in relation to kernel dry weight at 10 days post anthesis. Solid circles are triticales. Open circles are common wheat, durum wheat and rye. Data of KLASSEN (1970). Although dry weight of the seed was not measured at 0 days, unpollinated ovules weigh about 1 mg whereas the average dry weight of the triticale kernels at 10 days was 8.24 mg, the standard deviation was 2.77 mg and the range was 6.16 mg. It is therefore reasonable to suppose that variation in dry weight at 10 days post anthesis chiefly represents variation in the dry matter increment between 0 and 10 days. Correlations for triticale only. **: $p < 0.01$

Tab. 3 Correlation of the density of mature triticale kernels with dry weight of immature kernels at different dates after anthesis and with dry matter increments between these dates

Kernel dry weight		Kernel dry matter increment	
days after anthesis	correlation*)	days after anthesis	correlation*)
10**)	—0.870 **	10—24	+0.096 ns
24	—0.435 ns	24—38	—0.184 ns
38	—0.321 ns	38—52	—0.229 ns
52	—0.310 ns		

* Correlations calculated from data of KLASSEN (1970).

**) Although dry weight of seed was not measured at 0 days, unpollinated ovules weigh about 1 mg whereas average dry weight of the triticale kernels at 10 days post anthesis was 8.24 mg, the standard deviation was 2.77 mg and the range was 6.16 mg. It is therefore reasonable to assume that variation in dry weight at 10 days chiefly represents variation in the dry matter increment between 0 and 10 days.

**: $p < 0.01$; ns: non-significant.

Table 3). After this early period, however, changes in dry weight per kernel bear no significant relationship to shrivelling (*Table 3*). On the other hand shrivelling *is* related to the overall growth in volume. In triticale the maximum volume which a kernel attains during its development is negatively correlated with kernel density at maturity ($r = 0.710^{**}$, d.f. = 10, from SALMINEN

and HILL 1978). The fact that early growth in dry weight and overall growth in volume both correlate with kernel shrivelling suggests that they are related to each other. Initially the kernel grows in volume faster than it deposits starch. Consequently much of the early gain in dry weight probably goes into growth of the seed coat and general "infrastructure" so that a close relationship between early dry weight gain and maximum volume would not be surprising. It is also interesting that the association between rapid early gain in dry weight per kernel, large maximum volume during development and low density at maturity parallels the association between rapid endosperm division during the coenocytic period and kernel shrivelling at maturity which was noted previously. Similar coordination probably exists among many aspects of kernel growth. All these findings emphasize that kernel shrivelling is determined during early kernel development.

According to SISODIA (1973) large kernel size in triticale is associated with shrivelling. We suggest that a developmental program which leads to a large kernel volume will impose conditions on the early stages of development (rapid division rate; fast depletion of nutrients) which impair the subsequent ability of the kernel to fill itself (chromosome bridging; endosperm misdivision; aberrant nuclei leading to endosperm lesions). However, by this account we do not view large kernel size as a direct cause of shrivelling in triticale. In our opinion kernel size affects shrivelling only insofar as it correlates with chromosome bridging in the coenocytic endosperm.

Kernel Shrivelling and Carbohydrate Metabolism

In triticale, kernel density at maturity is significantly correlated with the percentage of kernel dry weight present as starch ($r = 0.764^*$, d.f. = 6, from KLASSEN 1970). Therefore kernel shrivelling involves a failure to deposit starch. Similarly, kernels of triticale that are destined to shrivel at maturity are poor sinks. They accumulate dry matter at lower rates and over shorter grain filling periods than the kernels of other triticales which remain relatively plump at maturity (HILL 1976, SALMINEN and HILL 1978). Also grain filling in triticale tends to cease at higher moisture contents than in wheat (KLASSEN 1970, SAARI 1977). However there is no evidence that a low supply of photosynthate leads to kernel shriveling in triticale.

The basic photosynthetic ability of triticale is about equal to that of bread wheat. While leaf area indices of triticale may be a bit low, the overall production of biomass by high yielding types is comparable to the best bread wheats (METZGER 1973, FISCHER 1973). Despite this large capacity for fixing dry matter, the demand of the grain sink for assimilates can be quite modest in triticale, especially where there is considerable partial sterility (SALMINEN and HILL 1978). Thus harvest index is generally lower in triticale than it is in wheat but despite the low demand for assimilates, kernel shrivelling is not affected. Neither harvest index nor the maximum aggregate volume of the grain sink correlate significantly with the degree of kernel shrivelling in tri-

ticale (*Table 4*). Treatments at anthesis involving crop thinning or removal of leaves showed that grain weight is limited in both wheat and triticale by the amount of carbohydrate available since the amount of dry matter translocated to individual kernels increased and decreased in response to a corresponding change in the supply of photosynthate (FISCHER 1973). However, kernel shrivelling in triticale was not materially affected by these changes in kernel weight and even a severe reduction in carbohydrate supply brought about through heavy shading did not induce wheat to shrivel in the way that triticale does (FISCHER 1973). Removal of florets to reduce the demand for photosynthate had no effect on kernel shrivelling in 'Rosner' triticale (P. J. KALTSIKES, unpublished data), actually reduced kernel weight in the shrivelled triticale line 6A190 and marginally increased kernel weight in the plump seeded line UM6531 and Manitou wheat (KLASSEN 1970). Sucrose supplied to the ear by the plant must pass through the vascular tissue of the peduncle on its way to the ear. No relationship was found between kernel shrivelling and the cross sectional area of phloem in the peduncle among advanced triticale lines although an indication of such a relationship was found for raw amphiploids (P. J. KALTSIKES, unpublished data).

Tab. 4 Correlations*) between sink size and kernel shrivelling in triticale

	Total maximum sink volume	Harvest index
Shrivelling index**)	—0.277 n.s.	+0.162 n.s.
Kernel density	—0.348 n.s.	+0.452 n.s.

n.s.: $p > 0{,}05$, $n = 12$.

*) Calculated from data of SALMINEN and HILL (1978).

**) Shrivelling index is the volume of the kernel at maturity divided by the maximum volume atteained by the kernel during development.

Carbohydrate enters the cereal kernel by one of two main routes. In wheat and barley, carbohydrate seems to enter the endosperm mainly as sucrose (BAXTER and DUFFUS 1973, JENNER 1974, SAKRI and SHANNON 1975) where it is probably converted into UDP-glucose and fructose by the enzyme sucrose synthase (PRESSEY 1969, TSAI et al. 1970, TURNER 1969). Sucrose synthase is a reversible enzyme that combines UDP-glucose or ADP-glucose and fructose into sucrose. In maize, most sucrose seems to be hydrolysed by invertase in the maternal tissues surrounding the endosperm so that carbohydrate enters the endosperm as glucose and fructose (SHANNON 1968, 1972, 1974, SHANNON and DOUGHERTY 1972). Although sucrose synthase was not detected in maize kernels before 12 days post anthesis it was present thereafter (TSAI et al. 1970). Therefore carbohydrate uptake into the endosperm of maize could proceed by either route after 12 days. In addition there is some evidence that sucrose hydrolysis may occur between the rachis phloem and the endosperm in wheat (SAKRI and SHANNON 1975). Consequently this difference in

the uptake and mobilization of sucrose between maize on the one hand and wheat and barley on the other may be one of emphasis rather than an absolute difference.

In triticale, the ability of the severely shrivelled line 6A190 to translocate photosynthetically fixed ^{14}C from the flag leaf into the kernel decreased after 23 days post anthesis compared to the plump seeded line 6A250 (HILL 1976). Similarly, the ability of cut heads of 6A190 excised 16 days after anthesis to take up labelled sucrose into the endosperm was about 10% lower than that of the plump seeded triticale UM6531 (KLASSEN 1970). Although this difference between UM6531 and 6A190 may be accounted for by the larger kernel size of UM6531, this line also partitioned more of the label it took up into the endosperm in preference to the pericarp than did 6A190. If these low transfers of label occurred because there was low demand for assimilates in the endosperm then sucrose concentrations should either build up in 6A190 or at least remain normal.

SALMINEN and HILL (1978) suggested that reduced conversion of sucrose into starch in shrivelled lines allowed the accumulation of soluble carbohydrates which raised the turgor of the young seed and drove cell expansion beyond the capacity to fill. Six days after anthesis sucrose levels in 6A190 were found to be high compared to 6A250 and wheat and rye (SAARI 1977). This was also true after making allowance for kernel size. An ontogenetic program to achieve large kernel size was noted previously to be associated with kernel shrivelling in triticale. Extra-high sucrose levels in the very young kernel of 6A190 to promote cell expansion could be one more aspect of this. In our view the problem in 6A190 is not so much the overall expansion of the kernel but rather the failure of the endosperm to keep pace with this expansion without partially breaking down. This interpretation is favored by data on percentage moisture content. Moisture content in triticale and rye is generally higher than it is in wheat (KLASSEN 1970, SAARI 1977, ZILLINSKY 1973) which might suggest overexpansion of the seed. However, within triticale itself average moisture content between 10 and 38 days after anthesis was not closely correlated with kernel density at maturity ($r = -0.412$, d.f. $= 6$, n.s. calculated from data of KLASSEN 1970). In addition the amount of sucrose in whole kernels of 6A190 subsequently dropped to very low levels about 14 days post anthesis and recovered about 22 days post anthesis compared to the levels in 6A250 and in wheat and rye (SAARI 1977). Consequently there is no evidence that reduced starch formation leads to sucrose build up in 6A190.

The soluble activity of sucrose synthase per kernel increased rapidly after fertilization in wheat, rye and triticale and peaked about the middle of the grain filling period (SAARI 1977). No qualitative differences in activity were found which might explain the fluctuating sucrose levels in 6A190 or why it shrivels so badly. When enzyme concentrations were re-expressed to allow for variation in kernel size the concentrations of sucrose synthase were found to be lower in triticale than in wheat. This difference is probably related to the high water content of triticale kernels and not closely related to shrivelling since there was no clear difference in the concentration of sucrose synthase

if only for a limited period. However the abrupt decline of sucrose levels within the kernel and their abrupt recovery a week later did not inflect the rate of dry matter gain in 6A190 which did not fall off until the end of the grain filling period (KLASSEN 1970, SAARI 1977). In conclusion there is no consistent evidence that under reasonable growing conditions the ability of the triticale plant to fix, to translocate or to mobilize photosynthate is a limiting factor which leads to kernel shrivelling. By elimination kernel shrivelling must represent a storage or "sink" limitation (FISCHER 1973).

Starch synthesis begins when free hexose units are converted into hexose-6-phosphates by the enzyme hexokinase. Various isomerase enzymes convert the different hexose phosphate units to glucose-1-phosphate. Glucose-1-phosphate is then converted to UDP-glucose or ADP-glucose by pyrophosphorylases (TSAI et al. 1970, TURNER 1969). UDP-glucose seems to function as the glycosyl donor for starch synthesis by a membrane bound glycosyl-starch transferase activity between 6A190 and 6A250. Adequacy of the sucrose synthase activities observed in 6A190 is also borne out by the fact that concentrations of its substrate sucrose were temporarily subnormal. Consequently these results suggest that the supply of sucrose to the endosperm of 6A190 might be inferior even that is most active in young endosperm whereas ADP-glucose is the substrate of a soluble starch-glycosyl transferase enzyme which becomes more important later on (BAXTER and DUFFUS 1971, SHANNON 1974, TSAI et al. 1970, TURNER 1969).

Total whole-kernel soluble activity of hexokinase increased throughout the period of grain filling in wheat, rye and triticale (SAARI 1977). There was a tendency for total soluble activity to increase in two phases. Activity per kernel increased during the first three weeks of kernel growth and later on around the fifth week of kernel growth with a plateau period in between. No qualitative differences in whole-kernel activity were noted which could be related to the shrivelling of 6A190. In the same way as for sucrose synthase the concentration of soluble hexokinase activity was lower in triticale and rye than in wheat. Initially the concentration of activity was higher in 6A190 than in 6A250 or rye but after about 2 weeks the variation in concentration of hexokinase paralleled the variation in mature kernel density (KLASSEN 1970). Durum and hexaploid wheat had the highest concentrations of hexokinase activity and the highest densities, 6A250 and rye were intermediate for both variables and 6A190 had the lowest concentration of hexokinase and the lowest kernel density (hexokinase concentrations calculated from figures of SAARI 1977; kernel densities from data of KLASSEN 1970). However, if the morphology of shrivelling in triticale is taken into account these differences in concentration of hexokinase are probably best accounted for by variation in the extent of normal endosperm tissues within the kernel rather than interpreted as a specific biochemical defect in the synthesis of polysaccharides.

ZHYLA and SHULYNDIN (1969) reported that shrivelling in triticale was accompanied by a deficiency in fine grained starch. They attributed this to a disturbance of starch synthesis in shrivelled triticales during the period when small starch granules are initiated. KLASSEN (1970) found that wheat generally

produces smaller starch granules than rye with most triticales falling between the two parental species. The exceptions were three triticale lines with *Triticum persicum* in their parentage, which had smaller starch granules equivalent in average size to those of wheat (KLASSEN and HILL 1971). However KLASSEN (1970) concluded that there was no relationship between the distribution of starch grain sizes and shrivelling in triticale. KALTSIKES and ROUPAKIAS (1975) noted that the first appearance of secondary starch granules was slightly delayed in most (but not all) of the shrivelled wheat-rye addition lines.

In conclusion there is no consistent evidence that inability of the triticale plant to meet the demand of the grain sink for starch precursors, or that low activity among the enzymes of the starch synthetic pathway contributes to the poor sink performance of shrivelled triticale kernels. Evidence of difficulties with the initiation of starch granules in shrivelled kernels is also contradictory.

One further possible explanation for low starch content of shrivelled triticale kernels could be premature lysis of previously deposited starch. Cells that are located around the margin of necrotic lesions or cavities within the endosperm or adjacent to abnormal sections of aleurone in kernels of 6A190 frequently contain eroded starch grains by 22 days after anthesis (DEDIO et al. 1975, DRONZEK et al. 1974, SIMMONDS 1974). Degradation of starch is initiated by the enzymes α- and β-amylases. The α-amylases are the enzymes responsible for the rapid scission of starch into soluble dextrins whereas β-amylases cut off maltose units from the free non-reducing ends of α 1—4 linked glucose polymers (THOMA et al. 1971).

In wheat and rye the α-amylase of developing whole kernels (α-I) is electrophoretically distinct from the additional α-amylases which appear after germination (α-II) (SILVANOVICH 1977). However, in the shrivelled triticale line 6A190 both α-I and α-II amylases are present throughout the development of the kernel. DEDIO et al. (1975) found that most of the α-amylase activity present in young kernels of wheat, rye and triticale is located in the pericarp with lower levels in the aleurone and endosperm. Particularly high levels were found in the pericarp and to a lesser extent the aleurone of shrivelled triticale lines compared to wheat or to plump seeded lines of triticale (DEDIO et al. 1975). In contrast there were no obvious differences among the levels of α-amylase in the young endosperm.

High levels of α-amylase activity in the pericarp would seem to be in the wrong position to damage endosperm starch. Consequently the significance of an association between high pericarp α-amylase activity and shrivelling is unclear. Higher than normal activities of α-amylase in the young aleurone of 6A190 could reflect contamination with this pericarp α-amylase during dissection rather than native aleurone α-amylase (DEDIO et al. 1975). Alternatively it could represent the premature activity of the α-II type amylases noted by SILVANOVICH (1977).

Levels of pericarp α-amylase activity decline as the kernel approaches maturity in all varieties of wheat, rye and triticale (DEDIO et al. 1975, SIMMONDS 1974). In wheat and rye there is also a tailing off in α-amylase activity in both the aleurone and endosperm before maturity (DEDIO et al. 1975).

However, in certain triticale lines, and especially in the shrivelled triticale line 6A190 there is enormous premature growth in α-amylase activity in the aleurone and endosperm starting about 23 days after anthesis (DEDIO et al. 1975, KLASSEN et al. 1971). This growth in α-amylase activity is associated with falling moisture content and falling levels of abscisic acid (R. D. HILL, personal communication). The beginning of the build up in α-amylase in 6A190 roughly coincides with the first appearance of eroded starch grains (DRONZEK et al. 1974, DEDIO et al. 1975, SIMMONDS 1974). Release of α-amylase within the maturing kernel is quite localized since the cells which contain eroded starch grains are distributed around the margins of endosperm lesions while neighboring cells may be full of sound starch grains (DEDIO et al. 1975, SIMMONDS 1974).

KLASSEN et al. (1971) reported a significant negative correlation among triticales between whole-kernel α-amylase activity at maturity and mature kernel density. This α-amylase activity is also negatively correlated with rapid growth of the kernel in the early stages (*Fig. 4*). Even within a single line of triticale highly shrivelled kernels contain more α-amylase activity than do plumper kernels (KLASSEN 1970). Consequently the association between shrivelling and α-amylase activity has been detected within the endosperm of individual kernels, among kernels within a triticale line and among triticale lines.

In wheat, rye and triticale levels of glucose in the very young kernel are relatively high. About 6 days after anthesis glucose comprises about 3 to 6% of the kernel by dry weight (SAARI 1977). It seems likely that much of this glucose comes from the digestion of starch reserves in the seed coat by pericarp α-amylase (DEDIO et al. 1975, SIMMONDS 1974). Thereafter the glucose content falls to much lower levels in all varieties (SAARI 1977). There is a tendency for both reducing sugar and glucose contents to be lower in wheats than in triticales (KLASSEN 1970, SAARI 1977) but much of this variation can be attributed to kernel size. However just before maturity there is a distinct increase in the level of reducing sugar in 6A190, probably released from endosperm starch by the growing α-amylase content (KLASSEN 1970). Like the rise in α-amylase content this increase in reducing sugar is closely associated with drying out of the kernel. A slight tendency for glucose levels to increase before maturity has also been found in 6A190 (SAARI 1977) although in this case sampling was not extended beyond about 40% moisture content. In triticale as a whole the concentration of reducing sugar at maturity is highly correlated with the α-amylase activity at maturity ($r = 0.903^{**}$, $df = 6$, KLASSEN 1970). However reduction of mature α-amylase content by prior application of cycocel produced no increase in kernel dry weight for the shrivelled triticale line 6A190 (KLASSEN 1970). This result might be expected for two reasons. Firstly, shrivelled triticale lines show maximum abnormality in α-amylase at the end of the grain filling period whereas their accumulation of dry matter is depressed from the beginning of the grain filling period (SALMINEN and HILL 1978). Secondly the total quantity of reducing sugars released in shrivelled triticale lines is not very great (KLASSEN 1970). Further-

more very high levels of α-amylase may fail to develop in triticale lines which shrivel badly at maturity (DEDIO et al. 1975).

It is suggested that premature increase of endosperm α-amylase activity is a frequent but not universal side effect of endosperm and aleurone lesions and that it is not a major determinant of shrivelling. On the other hand, high levels of α-amylase activity at maturity probably do account for the relatively high sugar levels, lack of dormancy and premature sprouting of particular triticale lines, especially under cool, wet conditions.

Kernel Shrivelling and Triticale Breeding

Although genetic improvement of kernel type by selection has been reported by many breeders the genetics of high test weight in triticale seems to be complex. Even in crosses between two lines of high test weight the frequency with which lines of comparable test weight are recovered is often low (CIMMYT review 1977). Secondly kernel shrivelling in triticale may be influenced by plant height. Thus ZILLINSKY (1973) reported that "attempts at visual selection for plump kernels tended very strongly to eliminate all the selections having dwarfing genes of Norin 10 origin". Thirdly shrivelling in triticale may also be associated with large kernel size (SISODIA 1973, SALMINEN and HILL 1978). Finally, there is the possibility that kernel development may be improved by reducing the heterochromatin content of the rye chromosomes (BENNETT 1977, GUSTAFSON and BENNETT 1976, J. P. GUSTAFSON, personal communication).

Reduction of kernel shrivelling in triticale is likely to result in the following changes. Firstly it should improve the sink or storage capacity of triticale and thereby improve its yield potential (FISCHER 1973). Second this increased storage of starch should raise test weight and yields of white flour obtainable from milling by increasing the starch content (KLASSEN 1970). Thirdly reduction in shrivelling will lower the protein content (LARTER et al. 1968, ZILLINSKY 1973, MCGINNIS 1973). Fourthly triticale flour produced from non-shrivelled lines should be lower in α-amylase (KLASSEN 1970) and therefore more useful in the making of leavened and noodle products (LORENZ 1972, LORENZ and WELSH 1974). Finally lowering α-amylase activity may also reduce the tendency of triticale to sprout in the ear prior to harvest, especially under cool wet conditions.

If triticale is envisaged as a feed grain then high protein is desirable and low flour yield and high α-amylase are irrelevant. In this case maximum yield per acre will be the most important breeding goal regardless of the degree of shrivelling (J. P. GUSTAFSON, personal communication). However, if triticale is envisaged as human food also, then the technological limitations associated with high α-amylase will reduce the versatility of triticale grain. Even though triticale may have particular agronomic advantages in certain environments we believe it will not become a well established crop until kernel shrivelling is largely eliminated.

Zusammenfassung

Zur Schrumpfkornentwicklung bei Triticale

Die nachstehenden Schlußfolgerungen werden aus der Übersicht der Schrumpfkornentwicklung bei Triticale gezogen:

1. Schrumpfen und geringe Sinkleistung der Triticale-Körner sind dadurch verursacht, daß einige Teile des Nährgewebes im Endosperm fehlen und das Wachstum des übrigen, normalen Gewebes mangelhaft ist. An Stelle der fehlenden Teile entstehen Hohlräume im Innern des Endosperms.
2. Die Hohlräume entstehen wahrscheinlich aus aberranten Kernen, die im cönozytischen Endosperm durch Teilungsfehler verursacht sind. Diese Fehler sind meistens während der Anaphase stattfindende Brückenbildungen bei den Roggenchromosomen.
3. Es ist bewiesen, daß die Schrumpfung der Körner durch ihr schnelles Wachstum in frühen Entwicklungsstadien und durch ihr großes, während der Entwicklung erreichtes Volumenmaximum verstärkt wird. Eine mögliche Beziehung zwischen dem schnellen Wachstum der Körner und gesteigerter Brückenbildung der Roggenchromosomen im Endosperm wurde angedeutet.
4. Es gibt keinen festen Beweis dafür, daß die Schrumpfkornbildung durch reduzierte Versorgung mit Stärkevorläufern oder durch unwirksame Aufnahme dieser Vorläufer verursacht ist.
5. Die vorzeitige Steigerung der α-Amylase-Aktivität im Endosperm kurz vor der Reife ist mit den Endosperm- und Aleuronschädigungen eng verbunden. Obschon vorzeitige Auflösung der Stärke durch α-Amylase zweifellos zur Depression der Stärkeanhäufung in geschrumpften Triticale-Körnern beiträgt, mag es vielleicht eher eine Nebenwirkung der Schrumpfung als ihre Hauptursache sein.

Der Kohlenhydratstoffwechsel bei Triticale verläuft meistens normal, wenn das Endosperm sich normal entwickelt. Ohne eine normale Entwicklung des Gewebes, das den Stoffwechsel durchführt, darf ein normaler Stoff- und Energiewechsel nicht erwartet werden. Wir schließen daraus, daß die Schrumpfkornbildung bei Triticale eher ein zytologischer und morphologischer Defekt als ein Defekt im Stärkestoffwechsel ist.

Unseres Erachtens ist weitere Forschungsarbeit auf den folgenden Gebieten notwendig: Die Beweise, daß fehlende Abnormität im Embryosack mit der Schrumpfung verbunden ist, sind unvollkommen. Der Mechanismus der Brükkenbildung bei Roggenchromosomen in cönozytischen Teilungen muß aufgeklärt werden. Das Verhältnis zwischen den cönozytischen, aberranten Kernen und den nachfolgenden Endospermabnormitäten wurde nur angenommen und muß weiter untersucht werden. Die drei in dieser Hinsicht interessierenden Punkte sind: die Gestaltung des „crease tunnel", die Verschiedenheit des Wachstums zwischen dem Endosperm und der Samenschale und der hohe Wassergehalt der Triticale-Körner. Schwankungen im Sucrose-Niveau und die

Weise, auf die Endospermläsionen eine vorzeitige Steigerung der α-Amylase bedingen, sind ebenfalls von Interesse.

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Note added in proof. New evidence on the origin of the endosperm aberrants and their effect on kernel development in triticale has been kindly communicated to us by Drs. J. P. GUSTAFSON and M. D. BENNETT. The near-complete loss of a large heterochromatin band from the $7R^L$ telomere of the triticale variety DRIRA has had the following effects. It reduced the frequency of aberrant nuclei in the coenocytic endosperm, and at the same time raised 1000 kernel weight, test weight and yielding ability. Consequently the influence of the $7R^L$ band on kernel shrivelling is firmly established. Simiar work on the $6R^S$ deletion in 'Rosner' should help establish whether all or only some of the major rye bands can incite the production of aberrant nuclei in the endosperm of triticale.

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