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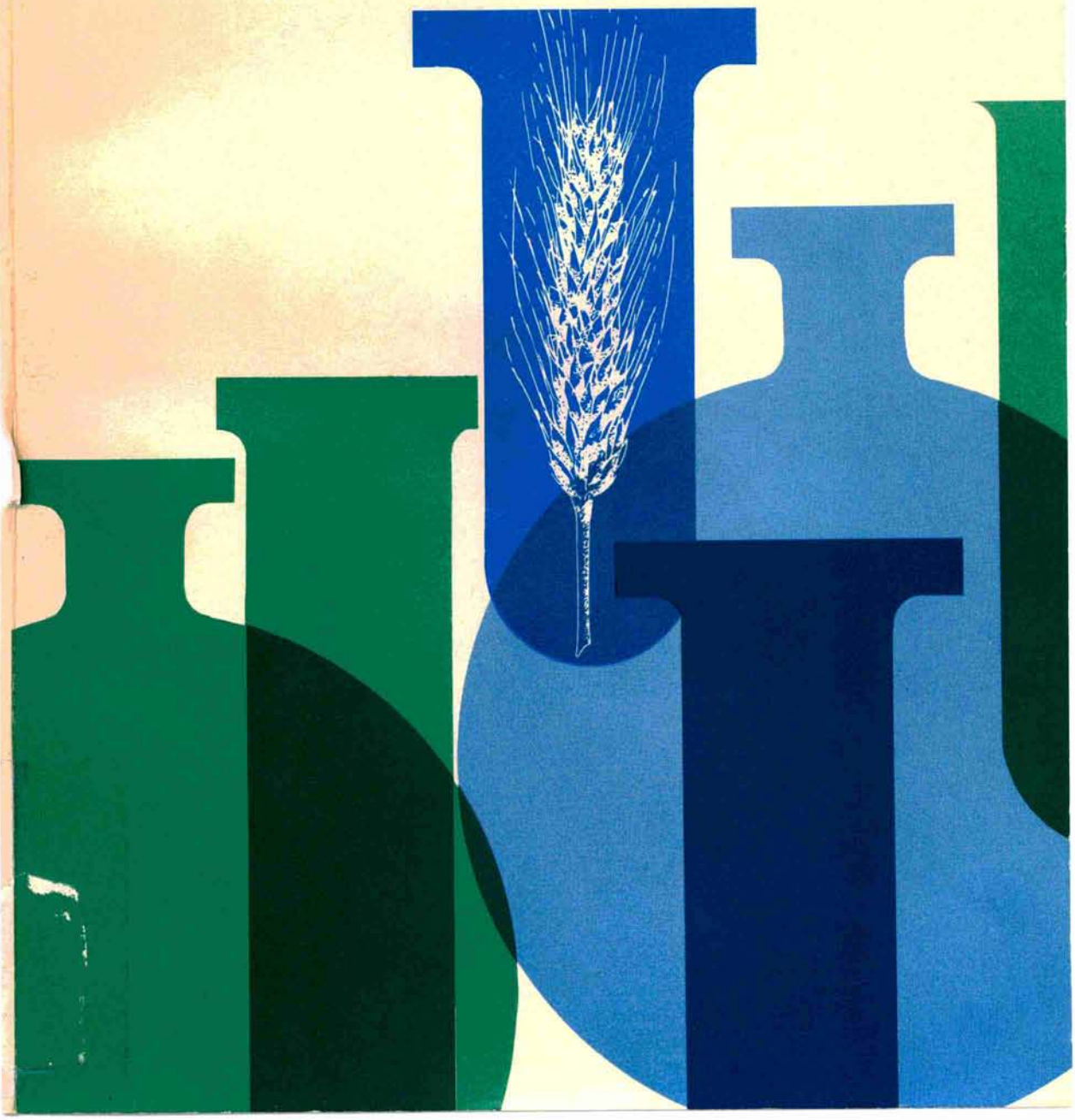

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Metabolic Factors Influencing Kernel Development in Triticale[1]

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Abstract Analysis for α-amylase activity in eight triticale cultivars varying in their test weight indicated a significant correlation ($r = -0.909^*$) between amylase activity and grain density. Starch content was positively correlated with test weight ($r = 0.746^*$). Starch deposition in some shrivelled cultivars was slower and the maximum starch content per unit of kernel volume was lower than in plump-seeded cultivars. Sucrose–^{14}C feeding experiments indicated that the shrivelled triticale cultivar, 6A190, was less efficient at transporting sucrose to the head than the plump cultivar, 6531. In addition, 6A190 deposited a larger proportion of the transported sucrose to the pericarp than 6531. Studies on the development of α-amylase during maturation showed that α-amylase activity in four triticale cultivars reached a maximum within the pericarp at approximately 12–15 days and declined to a minimum at approximately 20 days. Aleurone and endosperm α-amylase increased from approximately 20 days to a maximum at 28–32 days in all varieties except 6A190. In 6A190, α-amylase continued to increase as the grain matured, reaching levels that are characteristic of malted grains.

Résumé L'analyse de l'activité de l'alpha-amylase dans huit cultivars de triticale différant quant au poids lors des essais a indiqué une corrélation significative ($\nu = -0.909^*$). Chez certains cultivars à grain ratatiné, l'accumulation d'amidon etait plus lente et la teneur maximale en amidon était plus faible par unité volumétrique de grain que chez les cultivars à grains pleins. Des essais d'alimentation en saccharose-^{14}C ont indiqué que le cultivar de triticale ratatiné 6A190 transportait le saccharose vers l'épi moins efficacement que le cultivar plein 6531. De plus, chez 6A190, une proportion plus importante du saccharose transporté se déposait dans le péricarpe que chez 6531. L'étude du développement de l'alpha-amylase au cours de la maturation a montré que pour quatre cultivars de triticale son activité atteignait un maximum dans le péricarpe au bout de 12–15 jours environ puis déclinait jusqu'à un minimum pendant 20 jours à peu près. L'aleurone et l'alpha-amylase de l'endosperme augmentaient à partir du 20^e jour jusqu'à un maximum au bout de 28–32 jours pour toutes les variétés à l'exception de 6A190. Chez ce dernier, l'alpha-amylase continuait d'augmenter au fur et à mesure que le grain mûrissait, atteignant les niveaux qui caractérisent les grains maltés.

[1]Contribution No. 383 from the Department of Plant Science, The University of Manitoba, Winnipeg, Man., Canada R3T 2N2.

ALTHOUGH the synthesis of triticale has presented an opportunity to combine the high quality characteristics of wheat with the hardy competitive traits of rye, it has also introduced some of the poor quality characteristics of rye. Two of these characteristics are shrivelled kernels and high α-amylase activity. Kernel shrivelling in hexaploid triticales was first documented by Sanchez-Monge (1958). Reports from a number of sources indicate that mature triticale grain is higher in α-amylase activity than sound wheat (Müntzing 1963).

These and other observations have led us to investigate possible correlations between grain shrivelling and amylase activity in some triticale lines, the rationale being that either α-amylase caused breakdown of starch to produce shrivelling or that it was an indicator of pre-germination in the seed. This pre-germination could cause cessation of maturation processes resulting in seed shrivelling.

Materials and Methods

Plant Materials

For studies on relationship between α-amylase activity, grain density, and starch deposition during maturation, samples were grown under field conditions at Winnipeg during the summer of 1968. Spikes of all lines were tagged at the time of anther extrusion and six spikes of each line were harvested at each of seven different stages of maturity beginning 10 days after initiation of flowering and at weekly intervals thereafter.

For studies on the development of α-amylase during maturation, samples of triticale cultivars 6A190, 6A250, Beaver 'S' (E_1-68B-5N), and Kangaroo × UM940 'S' (X1005-10M-1Y-3M-3Y 0M) were grown in the greenhouse during the winter of 1972–73. At anthesis the spikes were tagged and from 6 to 42 days post-anthesis the spikes were harvested at 4-day intervals and stored at –20°C.

α-Amylase Analysis

Two methods of α-amylase analysis were used. In the studies using material from 1968, a viscometric method described by Tipples (1969) was used. In subsequent studies the method of MacGregor et al. (1971) was more sensitive and was used with some modification on material grown during 1972–73. The procedure was as follows.

At the time of α-amylase analysis five seeds from the middle of the spike were separated into pericarp, embryo, aleurone layer, and endosperm. Another three seeds were taken for α-amylase analysis of the whole seeds. The samples were homogenized with acetate buffer at pH 5.5 and after 2–3 h centrifuged in a clinical centrifuge. Ten millilitres of buffer were used for whole seed and for pericarp analysis, 5 ml for the aleurone layers and endosperms, except in certain samples with high α-amylase activity where more buffer was used, and 2 ml buffer for embryo analysis. After centrifugation, the supernatant was transferred to test tubes, being careful not to disturb the sediment. The method for α-amylase analysis was similar to that of MacGregor et al. (1971) with certain modifications. For α-amylase–dextrin incubations the volumes were reduced by half, as this was sufficient to obtain absorbance readings. A 1-ml sample was prepared from 0.2 ml of extract and 0.8 ml of acetate buffer. For triticale samples the incubation period was reduced to 5 min with two aliquots taken from each sample. With each set of unknowns, four samples containing 1 ml dextrin, 1 ml buffer (instead of the extract, which contains only small amounts of starch), and 5 ml of iodine solution were prepared as standards.

Soluble Sugars

Soluble sugars were extracted from 50- to 100-mg samples of ground grain with 3-ml portions of cold 80% ethanol. After evaporation of the ethanol the residue was resuspended in 10 ml of distilled water. Reducing sugars were measured in the supernatant by the ferricyanide method of Guinn (1967) with glucose as the standard.

Starch

Starch was estimated by a modification of the method of Donelson and Yamazaki

(1968). Twenty-milligram samples of ground grain were suspended in 4 ml distilled water and the starch gelatinized by placing the test tubes in vigorously boiling water for 2 min. The tubes were then cooled rapidly to 30°C and 5 ml acetate buffer (pH 4.7) was added and placed in a 30°C water bath. One millilitre of α-amylase solution (0.020 g Mann α-amylase; 19,900 BU/g) was added and incubation carried out for 1.5 h and the enzymatic hydrolysis was stopped by the addition of 1 ml of 50% trichloracetic acid. After neutralization with NaOH, centrifugation, and appropriate dilution, reducing sugars produced by the hydrolysis were measured using the Guinn (1967) method. Pure Lintner starch (Fisher Scientific Co.) was used as the standard and corrections were made for free reducing sugars present in the samples before hydrolysis.

Results and Discussion

In the initial studies (Klassen 1970; Klassen et al. 1971) seven hexaploid triticale lines and one octoploid variety were analyzed for a number of parameters that could be related to shrivelling. Some of the results of these experiments are summarized in Table 1. Test weight varied from about 54 to 65 kg/hl for the triticales. This is considerably lower than Stewart 63 and Manitou wheats, which were included as controls. Some of the varieties approached Prolific rye in test weight. Seed volume has been included to give an indication of seed size since 1000 kernel weight does not always reflect seed size where severe shrivelling is present. For example, 6531 and 6211.2 have approximately the same seed volume, yet their 1000 kernel weight is 52.5 and 43 g, respectively. There was a highly significant statistical correlation of -0.909^{**} between test weight and α-amylase activity among the lines studied. A 15-fold variation in the amylase activity of the triticales was observed with some varieties having almost 50 times the activity of the hexaploid wheat. There was no correlation between reducing sugar content and test weight but the level of reducing sugar in the seed did reflect the α-amylase activity. Starch content was positively correlated with test weight ($n = 0.746^{*}$) indicating that the loss in test weight was probably due to a decrease in the starch content of shrivelled seed.

The rate of starch deposition during maturation does vary from variety to variety. For example, Fig. 1 shows the starch content of four triticale lines and Manitou wheat at various stages of development. The initial slopes of the curves of the two lines of lowest bushel weight, 6211.2 and 6A190, are smaller than 6531 and 6A320, indicating that they have a slower rate of starch deposition. In addition, the maximum starch content per

TABLE 1. Characteristics of the mature grain of experimental lines.

Sample	Test weight (*kg/hl*)	Seed volume (*cc*)	α-Amylase[a] (*units/kernel*)	Reducing sugars (*mg/g*)	Starch (%)
Manitou	78.2	0.023	0.028	1.23	57.2
Stewart 63	81.0	0.034	0.238	1.87	56.6
Prolific	66.4	0.024	0.409	2.39	57.2
8A92	64.2	0.029	0.408	1.54	54.2
6531	65.0	0.041	0.762	2.01	55.3
6A250	59.6	0.020	0.084	2.32	57.1
6A320	59.6	0.031	0.292	1.90	51.5
64563	61.0	0.031	0.694	2.39	51.9
6517	57.8	0.033	1.673	3.33	54.0
6A190	54.4	0.039	2.001	3.59	51.9
6211.2	54.3	0.040	1.872	4.21	49.1

[a]Determined by the viscometric method of Tipples (1969).

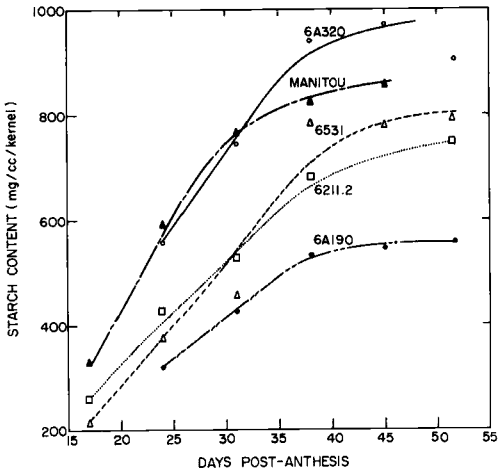

FIG. 1. Starch content in some triticale cultivars and Manitoba wheat as a function of kernel development.

unit of kernel volume of 6211.2 and 6A190 is lower than the varieties 6531 and 6A320. These latter two varieties have superior seed characteristics. Manitou starch content reaches a plateau level 5–10 days earlier than triticale. The results in Fig. 1 have been expressed on a seed volume basis to assess the ability of the particular variety to fill the available space in the endosperm. Thus, Manitou, a wheat variety that has relatively small seeds, does not deposit as much starch per seed as 6A190, a large-seeded triticale. However, when considered on a volume basis the synthesis of Manitou is sufficient to completely fill the volume within the endosperm whereas 6A190 is extremely shrivelled.

In addition to the reduced levels of starch deposition in shrivelled varieties, the rate of sucrose transport to the kernels may also be limiting. This is suggested by feeding experiments in which 16-day-old heads of 6A190 and 6531 were allowed to take up ^{14}C-labelled sucrose via the cut stems. The radioactivity incorporated into the endosperm and pericarp–testa was determined and the results are summarized in Table 2. Considering the total radioactivity incorporated, the cultivar 6A190 is 6% less efficient at transporting sucrose to the kernel when compared to 6531. In terms of sucrose incorporation into the endosperm, 6A190 transports 10% less sucrose than 6531. In addition, 6A190 directs a greater proportion of the incorporated radioactivity into the pericarp–testa than 6531. Thus, at the stage where endosperm starch synthesis is occurring most rapidly the shrivelled triticale cultivar is transporting sucrose at a slower rate and converting a greater proportion of the transported radioactivity into nonstorage materials in the pericarp–testa.

TABLE 2. Sucrose–^{14}C incorporation into grain of excised 16-day-old heads of two triticale lines.

	Incorporation (*dpm*)		
	6531	6A190	6A190 as % of 6531
Endosperm	26,989	24,481	90.7
Pericarp–testa	10,169	10,558	104.1
Total	37,158	35,039	94.3

As a follow-up to the earlier observations on a relationship between α-amylase activity and kernel shrivelling, we recently studied the localization of α-amylase in the tissue during the maturation period in four varieties of triticale. Two of the triticale varieties, 6A190 and Beaver 'S' (E_1-68B-5N), are shrivelled whereas the other two, 6A250 and Kangaroo × UM940, are plump. We included the Beaver and Kangaroo lines because they are advanced lines compared to 6A190 and 6A250, which are new amphiploids. The changes in the total α-amylase in these samples during development are shown in Fig. 2. All varieties have a characteristic peak of activity at about 12–15 days, which declines to a minimum at approximately 20 days. There is a second peak of enzyme activity at 28–32 days in all varieties, except 6A190, followed by a decline as the grain matures. The second burst of activity is unique in 6A190 since it continues to increase as the grain matures reaching levels characteristic of malted grains. The α-amylase activity in the shrivelled varieties is higher than that in the nonshrivelled varieties even though the difference is not pronounced in the Beaver line.

The localization of α-amylase activity in the tissue of 6A190 is shown in Fig. 3. The

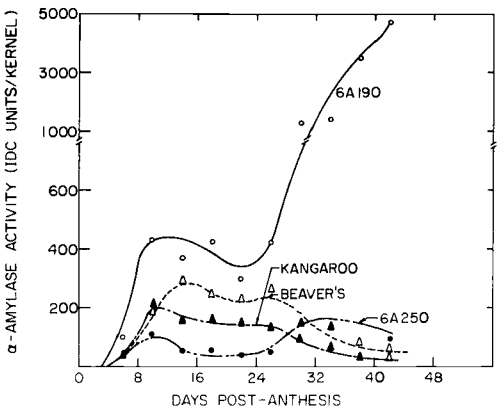

FIG. 2. Variation in total α-amylase of selected triticale lines at various stages of seed development.

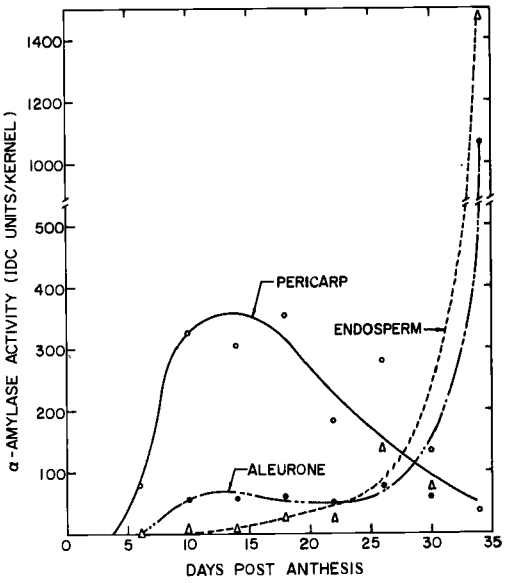

FIG. 3. Variation in the pericarp, endosperm, and aleurone α-amylase of triticale cultivar 6A190 at various stages of development.

peak of enzyme activity observed at approximately 12–15 days in the whole seed is associated almost entirely with the pericarp. This agrees with earlier work on the development of α-amylase in barley (MacGregor et al. 1972) and in wheat (Kruger 1972). This enzyme may degrade pericarp starch during early seed development providing nutrients for endosperm synthesis. As maturation proceeds, the pericarp amylase declines, and at approximately 22 days, the α-amylase activity within the aleurone and endosperm tissue of 6A190 increases dramatically. The development of α-amylase activity within the aleurone layer is normally associated with germination processes and may indicate the onset of precocious germination in this variety.

The development of α-amylase activity in the other three triticale lines is shown in Table 3. Pericarp α-amylase activity in all

TABLE 3. Distribution of α-amylase in triticale kernels during development.

Days after anthesis	Amylase activity[a]								
	6A250			Beaver 'S'			Kangaroo × UM 940 'S'		
	Pericarp	Aleurone	Endosperm	Pericarp	Aleurone	Endosperm	Pericarp	Aleurone	Endosperm
	(IDC *units/kernel*)								
6	24	5	0	36	15	3	27	19	3
10	66	12	6	130	38	8	148	34	12
14	36	5	7	240	26	15	138	19	6
18	61	10	0	216	39	8	142	43	13
22	22	11	5	223	27	20	143	8	19
26	37	10	7	256	46	20	74	38	30
30	20	14	10	70	50	50	82	21	7
34	4	27	8	58	31	40	34	20	13
38							18	10	6

[a]Determined by the McGregor et al. (1971) method.

lines is maximal during the period from 10 to 20 days. However, in the Beaver line this activity is higher and more prolonged than in the other two lines. Aleurone and endosperm activity is relatively low in all lines throughout the development period when compared to 6A190. Thus, the presence of amylase activity in association with shrivelling, although still evident in the pericarp tissue, is not as dominant in the Beaver line. The lack of a significant endosperm and aleurone α-amylase in the shrivelled Beaver 'S' line may indicate that shrivelling is not associated with precocious germination in this cultivar. Confirming this will require an analysis of events in the germination process that normally occur prior to the appearance of α-amylase.

In general, our results indicate that shrivelled cultivars of triticale have higher kernel α-amylase levels both at maturity and during their development than non-shrivelled lines. The rate of starch deposition in shrivelled cultivars relative to kernel volume is slower than in plump-seeded cultivars. Studies with 6A190 suggest that nutrient transport to the head may also be limiting in shrivelled cultivars.

References

DONELSON, J. R., AND W. T. YAMAZAKI. 1968. Enzymic determination of starch in wheat fractions. Cereal Chem. 45: 177–182.

GUINN, G. 1967. An ultrasensitive chemical test for quantitative chromatography of sugars. J. Chromatogr. 30: 178–182.

KLASSEN, A. J. 1970. An investigation of kernel shrivelling in triticale. Ph.D. Thesis. Univ. of Manitoba, Winnipeg, Man.

KLASSEN, A. J., R. D. HILL, AND E. N. LARTER. 1971. Alpha-amylase activity and carbohydrate content as related to kernel development in triticale. Crop Sci. 11: 265–267.

KRUGER, J. E. 1972. Changes in the amylases of hard red spring wheat during growth and maturation. Cereal Chem. 49: 379–390.

MACGREGOR, A. W., A. G. GORDON, W. O. S. MEREDITH, AND L. LACROIX. 1972. Site of α-amylase in developing barley kernels. J. Inst. Brew. 78: 174–179.

MACGREGOR, A. W., D. E. LABERGE, AND W. O. S. MEREDITH. 1971. Changes in barley kernels during growth and maturation. Cereal Chem. 48: 255–269.

MÜNTZING, A. 1963. Some recent results from breeding work with ryewheat, p. 167–178. *In* Recent plant breeding research. Svalof 1946–1961.

SANCHEZ-MONGE, E. 1958. Hexaploid triticale. Proc. 1st Int. Wheat Genetics Symp., Univ. of Manitoba, Winnipeg, Man. p. 181–194.

TIPPLES, K. H. 1969. A viscometric method for measuring α-amylase activity in small samples of wheat and flour. Cereal Chem. 46: 589–598.