Grain Shrivelling in Secondary Hexaploid Triticale. I. Alpha-Amylase Activity and Carbohydrate Content of Mature and Developing Grains[1]

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Cereal Chem. 59(6):454–458

ABSTRACT

Eight secondary hexaploid triticale cultivars of known wheat parentage and rye chromosomal composition, grown under uniform conditions at Cd. Obregon, Sonora, Mexico, during the winter of 1977–1978, were evaluated for α-amylase activity and carbohydrate content. Grain from each cultivar was classified as plump, medium shrivelled, and highly shrivelled. All triticale whole grain and flour samples had higher α-amylase activity than wheat or rye. α-Amylase activity and starch content of mature grain varied with the extent of grain shrivelling, whereas total soluble sugars and reducing sugars were unrelated to grain shrivelling in secondary hexaploid triticale. Compositional changes during seed development from the three grain classes of the triticale cultivar Rahum indicated that the factors producing grain shrivelling also affect the α-amylase activity in the grain. Changes in water and starch contents indicated that the highly shrivelled grains reached physiological maturity earlier than did the plump grains. Total and reducing sugars did not reflect the high α-amylase activity of highly shrivelled grains, suggesting that the grain can eliminate excessive levels of soluble carbohydrate, possibly by catabolizing or relocating mechanisms.

The significant success achieved with triticale is overshadowed by the persistance of grain shrivelling, an undesirable characteristic that results in low grain test weights and low flour yields.

Bennett (1974) and Bennett et al (1975) suggested that nuclear instability in triticale at meiosis and in developing endosperm may result from the interaction of the parental wheat and rye genomes that control development at different rates in the hybrid. Kaltsikes and Roupakias (1975) and Kaltsikes et al (1975) proposed that this genomic interaction may result in certain aberrant nuclei forming at early stages of endosperm development as a result of late-replicating heterochromatic rye chromosome segments. Bennett et al (1975) found that most of the aberrant endosperm nuclei formed bridges between nuclei and, less frequently, between micronuclei, due to endomitosis (no spindle formed). They also observed that aberrant endosperm nuclei were positively related to the degree of grain shrivelling in triticale.

Biochemical and physiological aspects of grain shrivelling in triticale also have been studied. According to Klassen et al (1971), triticale has a short dormancy period resulting in premature α-amylase activity and precocious germination that may produce kernel shrivelling. They found, with one exception, that α-amylase activity correlated highly with the degree of grain shrivelling. Similar studies on different triticale genotypes (Lorenz and Welsh 1976, Noll 1977, Rao et al 1976, Singh et al 1978) showed that α-amylase activity decreased during the early stages of maturation but increased rapidly at about 50–55% moisture or 22 to 28 days post-anthesis (p.a.) in triticale lines with extensive kernel shrivelling. However, Chojnacki et al (1976) found that, although grain shrivelling caused high α-amylase activity in several triticale lines, the experimental materials had heterogeneous activity changes in α-amylase. They concluded that triticale may be bred with α-amylase levels similar to those in rye and suggested that high α-amylase activity was usually unrelated to grain shrivelling and precocious germination. In accordance with this, Agrawal (1977) found that a triticale line with shrivelled grains had α-amylase activity nearly as low as that of wheat, whereas the plump-grained triticale had higher α-amylase activity at certain stages of grain development than the shrivelled-grained triticale. He concluded that amylases and, hence, sprouting were not related to grain shrivelling in the materials he studied.

On the other hand, the levels of some carbohydrates have been correlated to the degree of shrivelling in triticale grain. Klassen et al (1971) observed that starch accumulation during grain development terminated earlier in shrivelled-grain triticales than in plump-grain triticales and remained constant until maturity. They also observed that reducing sugars remained higher at maturity in the more shrivelled-grain triticales than in the plump-grain triticales. Similar results were obtained by Singh et al (1978), who found that total alcohol-soluble sugars were higher when the starch content was lower in shrivelled-grain triticale. Noll (1977) observed similar relationships among starch, total soluble sugars, and grain shrivelling in triticale, but no significant difference in reducing sugars between shrivelled- and plump-grain triticales. Hill et al (1974) fed sucrose-C^{14} to two triticale cultivars (one plump and one shrivelled) and determined that the shrivelled triticale was less efficient than the plump triticale in transporting sucrose to the head. The shrivelled cultivar deposited more transported sucrose to the pericarp than did the plump one. In addition, the rate of starch deposition was slower in the shrivelled triticale than in the plump one. They concluded that nutrient transport to the head may also be involved in producing grain shrivelling, which agreed with observations by Jenner and Rathjen (1972a, 1972b) of an active barrier against nutrient transport in some wheats.

Because the many ideas and hypotheses about triticale grain shrivelling conflict, and because genetic and environmental differences confound the data, we designed this research to test earlier hypotheses and to unify the concepts of seed shrivelling. We investigated carbohydrate composition and α-amylase activity and their possible relationships with grain shrivelling in eight secondary hexaploid triticale cultivars of known wheat parentage and rye chromosomal composition and in one cultivar during grain development.

MATERIALS AND METHODS

Mature Grain Study Materials

Secondary hexaploid triticale, wheat, and rye samples were grown under uniform environmental conditions at Cd. Obregon, Sonora, Mexico during the winter of 1977–1978 as part of the International Maize and Wheat Improvement Center's (CIMMYT) triticale yield trials. The triticale lines, which are advanced products of intercrossing and selection, were chosen to obtain material with different degrees of grain shrivelling and to minimize genetic and environment variables. Varieties, or cross names, and identification numbers are presented in Table I. Grains from each triticale cultivar were categorized as plump, medium shrivelled, and highly shrivelled. To obtain whole-meal flours, we ground the samples as received in a Udy-Cyclone pulverizer to pass a 0.5-mm screen. Flours were obtained from triticale samples tempered at 12.5% moisture for 24 hr, wheat samples at 15%

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0009-0352/82/06045405/$03.00/0

moisture for 24 hr, and the rye sample at 14% moisture for 24 hr. All samples were milled in a Brabender Quadrumat Senior experimental mill to pass a 10-xx mesh sieve.

Developing Grain Study Materials

Seeds of the three grain classes of the triticale cultivar Rahum were grown separately under controlled environmental conditions. As the heads emerged, each was bagged, and the anthesis date recorded. The spikes were harvested in the afternoon at each of five different stages of maturity (7, 14, 21, 28, and 35 days post-anthesis [p.a.]). The grains were removed from three to five spikes, immediately weighed, and freeze-dried for 36 hr to obtain the moisture content of the samples. The freeze-dried samples were ground initially in a Super Junior Moulinex coffee grinder and pulverized finely in a Wig-L-Bug dental amalgamator.

Moisture, Protein and Ash

Moisture, protein, and ash were determined in duplicate samples by the official methods (13.004, 38.012, and 13.006, respectively) of the AOAC (1965).

α-Amylase Activity

α-Amylase activity was determined by the method of Barnes and Blakeney (1974), which uses partially hydrolyzed potato starch crosslinked by 1,4 butandioldiglycidether and labeled with Cibacron blue by covalent bonds. This substrate, available commercially as Phadebas tablets (Pharamacia Laboratories, Inc., Piscataway, NY), is resistant to β-amylase degradation and liberates water-soluble dye-labeled products on α-amylase hydrolysis.

A partially purified triticale (P-10) α-amylase was prepared according to Greenwood and Milne (1968), and its activity determined. Ten milligrams of the partially purified α-amylase was dissolved in 10 ml of 0.1*M* acetate buffer (pH 5.5) containing 0.001*M* $CaCl_2$. One milliliter of the properly diluted enzyme solution was incubated in the presence of a starch solution and prepared according to Strumeyer (1967) at 30° C for 10 min.

Reducing products were measured by the Nelson colorimetric copper procedure as described by Robyt and Whelan (1968). The activity was determined as international enzyme units (EU). One EU is equal to 1 μ*M* of maltose produced for 1 ml of enzyme solution in 1 min. α-Amylase activity was obtained by converting absorbance values to EU by using the calibration curve from the action of partially purified triticale (P-10) α-amylase in the Phadebas substrate.

Starch and Total Soluble Sugars

Starch and total soluble sugars were determined with Dreywood's anthrone reagent as described by McCready et al (1950). Glucose (μg) multiplied by 0.92 was used to convert to starch (μg).

Reducing Sugars

Reducing sugars were determined with the Nelson procedure (1944).

RESULTS AND DISCUSSION

All triticale lines studied contained 2n=6X=42 chromosomes[2] and were advanced secondary hexaploid triticales as products of intercrosses among triticale and between triticales and hexaploid wheats.

Composition of Mature Grain and Flour

Protein and Ash Content. Moisture, protein, and ash content of the experimental grains are given in Table II. Triticale protein varied from 11.8 to 13.7% with an average of 12.6%, whereas wheat (P-8 and P-9) and rye (P-11) samples contained 14.8, 13.0, and 12.6% protein, respectively. When grain samples were converted to flour, an average drop of 1.95% protein occurred in triticale, compared to 1.8, 0.8, and 4.6%, respectively, for wheat (P-8 and P-9) and rye (P-11). Thus, flour protein among triticales varied from 9.6 to 11.7%, with an average of 10.6%, whereas wheat (P-8 and P-9) and rye flour samples contained 13.8, 12.2, and 8.0%, respectively. Ash content of triticale (except P-6 and P-7) and of rye was slightly higher than that of wheat flours. Protein and ash values indicated that triticale endosperm is intermediate between wheat and rye.

α-Amylase Activity. Grain and flour α-amylase activity varied widely among triticales but usually was many times higher than in wheat or rye (Table III), which agrees fully with values reported by Klassen et al (1971), Dedio et al (1975), Lorenz and Welsh (1976), Rao et al (1976), and Singh et al (1978). The flour samples (except P-6 and P-11) had lower α-amylase activity than the corresponding grain sample, probably because most of the grain α-amylase activity is in the aleurone and endosperm cells immediately below the aleurone (Dedio et al 1975) and was not obtained in our low-extraction triticale flour (as indicated by ash content, Table II). The slightly higher α-amylase activities in P-6 and P-11 flour than in grain indicated that α-amylase activity in the aleurone was lower than in the endosperm, as Dedio et al (1975) and Simmonds and Campbell (1976) also observed when studying the rye cultivar Prolific.

Although a relationship between α-amylase activity and the

TABLE I
Triticale, Wheat, and Rye Samples

Variety No.	Variety or Cross Name	Identification No.
Triticale		
424	Rahum	P-1
430	Mapache	P-2
823	PM 28 Bulk-Cml "s" X-21349-2N-OY	P-3
827	1A-M_2A × Pi62/Bgl "s" X-116304-110-1M-OY	P-4
1628	Drira-Arm "s" X-21367-4N-OY	P-5
2427	IRA^2 × M_2A X-11308-B-2M-3Y-2Y-4M-OY	P-6
1303	1A-Kla × Cal X-14920-2Y-OM	P-7
1401	Bgc-Bulk e2 X-11066-A-6M-100Y-101B-100Y-OY	P-10
Wheat	Calidad	P-8
	Hermosillo	P-9
Rye	Snoopy	P-11

TABLE II
Moisture, Protein, and Ash Content of Triticale, Wheat, and Rye[a]

Sample No.	Grain[b] Moisture (%)	Grain[b] Protein (%)	Flour[c] Moisture (%)	Flour[c] Protein (%)	Ash (%)
P-1	10.2	12.4	12.2	10.8	0.53
P-2	9.7	12.3	11.8	10.7	0.52
P-3	9.4	12.7	12.0	10.6	0.57
P-4	10.0	11.8	12.2	9.6	0.57
P-5	9.6	12.0	12.0	10.3	0.56
P-6	10.0	12.6	12.0	10.0	0.46
P-7	9.7	13.5	12.0	11.3	0.46
P-8	10.6	14.8	13.6	13.0	0.47
P-9	10.3	13.0	14.3	12.2	0.39
P-10	10.4	13.7	12.6	11.7	0.53
P-11	9.4	12.6	11.8	8.0	0.51

[a] Protein and ash on a 14% moisture basis.
[b] Grain protein (N × 5.83).
[c] Triticale and rye flour protein (N × 5.83); wheat flour protein (N × 5.7).

[2] K. Mujeeb. 1978. Personal communication.

TABLE III
Biochemical and Chemical Composition of Grain and Flour Samples[a]

Sample No.	Grain				Flour			
	α-Amylase Activity[b]	Starch (%)	Total Soluble Sugars (%)	Reducing Sugars (%)	α-Amylase Activity[b]	Starch (%)	Total Soluble Sugars (%)	Reducing Sugars (%)
P-1	283.1	59.6	3.7	0.20	163.1	79.3	2.1	0.20
P-2	179.4	59.4	4.1	0.23	102.0	74.0	2.1	0.27
P-3	211.0	59.0	4.8	0.23	128.1	77.8	2.2	0.23
P-4	181.7	62.3	3.9	0.17	133.1	79.0	2.0	0.16
P-5	613.9	59.2	5.2	0.24	418.8	73.9	3.6	0.23
P-6	46.7	65.3	4.8	0.19	54.6	74.0	2.6	0.21
P-7	212.6	57.0	5.6	0.25	105.2	70.9	5.0	0.34
P-8	2.4	58.5	2.6	0.16	0.8	72.5	1.8	0.28
P-9	1.4	66.2	3.0	0.20	0.9	75.1	1.8	0.31
P-10	182.3	61.2	4.2	0.16	131.1	68.2	2.7	0.25
P-11	2.3	54.5	5.0	0.21	7.3	76.2	4.4	0.16

[a] Dry weight basis.
[b] International enzyme units.

TABLE IV
Ranking of the Triticale Samples Based on Frequency of Plump, Medium Shrivelled, and Highly Shrivelled Grains[a]

Sample	Grain Class[b]			Grain Quality Ranking[c]
	a (%)	b (%)	c (%)	
P-1	39.0	47.6	13.4	3
P-2	28.6	56.5	14.9	4
P-3	18.0	62.3	19.7	6
P-4	20.0	69.9	10.1	5
P-5	14.2	65.6	20.2	8
P-6	49.8	42.8	7.4	1
P-7	46.2	44.1	9.7	2
P-10	17.1	56.8	26.1	7

[a] Average percent of three replicate determinations.
[b] a = plump, b = medium-shrivelled, c = highly shrivelled.
[c] 1 = highest frequency, and 8 = lowest frequency of plump grains.

extent of grain shrivelling in triticale has been suggested, we found no relationship in these advanced secondary hexaploid triticales. Because plump to highly shrivelled grains could be observed in each cultivar bulk sample, a subjective visual classification of plump, medium shrivelled, and highly shrivelled grains (classes a, b, and c, respectively) within each of the eight triticale cultivars was established, and grain quality was ranked by the frequency of plump grains in each cultivar (Table IV). No evident relationship between α-amylase activity and extent of grain shrivelling could be observed, even though whole meal and flour triticale samples might not have included the grain classes in the same proportions estimated by visual selection. To draw firm conclusions, we analyzed each of the three grain classes of all eight triticale cultivars (P-la, P-lb, P-lc, P-2a–P-10c, Table V). The results were consistent within triticale cultivars in that the plumpest grain had the lowest α-amylase activity. The α-amylase activity of the highly shrivelled class c varied widely among triticales. Although the lowest α-amylase activity of any highly shrivelled classes was higher than that of any a or b class of all eight triticale cultivars, this pattern probably resulted from the fact that the amount of starch endosperm is less in the highly shrivelled grains than in the plump ones, thus resulting in a smaller ratio of starchy endosperm to aleurone, and, consequently, higher α-amylase concentration. These results provide additional evidence to eliminate any contention that α-amylase activity is the major factor influencing grain shrivelling in triticale, which supports fully the earlier results of Klassen et al (1971) and Agrawal (1977).

Starch. Triticale grain and flour starch contents ranged from 57.0 to 65.3% and from 68.2 to 79.3%, respectively (Table III). The starch content of wheat grain and flour fell within the respective range of starch content of triticale. (P-9 grain at 66.2% was slightly high.) Rye grain had the lowest starch content, whereas rye flour starch was within the triticale flour range. No relationship between starch content (in grain and flour) and extent of shrivelling could be established using ranked triticale (Table IV).

When the visually categorized classes of the eight triticales were analyzed, the starch content was observed consistently higher in the plump—a class within a cultivar (Table V). These observations may be explained partially by collapses to fill empty spaces in the endosperm (Simmonds 1974), by variations due to packing of contents within the endosperm, by variations in both grain size and total dry matter accumulation (Salminen and Hill 1978), as well as by variations in α-amylase activities among the triticales studied. The relationship between starch content and extent of grain shrivelling agrees with the results of Klassen et al (1971), Hill et al (1974), and Noll (1977) but is contrary to Agrawal's (1977) results comparing only two triticale lines (one plump and one shrivelled).

Total Soluble Sugars. Total soluble sugars ranged from 3.7 to 5.6% and from 2.0 to 5.0%, respectively, for triticale grain and flour (Table III). Wheat grain and flour had lower total soluble sugars than the respective triticale materials, whereas rye grain and flour total soluble sugars fell within the respective ranges of total soluble sugars in triticale. In ranked triticales (Table IV), no relationship between total soluble sugars (in grain and flour) and extent of grain shrivelling was found. When the three classes of all eight triticales were analyzed, the highly shrivelled grain class within cultivars exhibited the highest total soluble sugars content, although in some cultivars (eg, P-3, P-5, and P-10), the highly shrivelled grain class did not have considerably higher total soluble sugars than the plump one (Table V). This relationship was not observed consistently for the plump and medium shrivelled grain classes. Similar results were obtained by Noll (1977). Therefore, we concluded that total soluble sugars is not a characteristic that indicates strongly the extent of grain shrivelling in triticale.

Reducing Sugars. Reducing sugars in triticale grain and flour ranged from 0.16 to 0.25% and from 0.16 to 0.34%, respectively (Table III). Wheat and rye (grain and flour) fell within the respective range of reducing sugars observed in triticale. When the grain classes of all the triticales were analyzed and reducing sugars contents compared within the grain classes, the differences observed were not large enough to be considered significantly related to the extent of grain shrivelling (Table V). Noll (1977) also reported no significant differences in reducing sugars among shrivelled and plump-grained triticales.

Compositional Changes During Grain Development as Influenced by Grain Shrivelling

Changes in composition during the development of grains, derived from three different grain classes (P-1a, P-1b, and P-1c) of the triticale cultivar Rahum, are presented in two ways in Table VI. For comparison, the results are discussed as milligrams per kernel and EU per kernel.

Water. Water content changed in a similar manner in all grain samples during the first 28 days p.a. Loss of water was first manifested at 21 days p.a., as Agrawal (1977) observed. From 29 to 35 days p.a., grains from P-1a and P-1b continued to lose water at about the same rate. In contrast, grains from P-1c lost water rapidly to levels characteristic of mature grains after harvest. Thus, at 35 days p.a., the highest water content corresponded to grains from P-1b (15.5 mg per kernel) and the lowest to grain from P-1c (2.2 mg per kernel).

α-Amylase Activity. In all grain samples, α-amylase activity increased from anthesis to 14 days p.a., then decreased during the next seven days. However, the rate of α-amylase activity increase varied among the samples. At 21 days p.a., the highest α-amylase activity occurred in grains from P-1c (3.97 EU per kernel), and the lowest corresponded to P-la (3.58 EU per kernel). From 21 to 28 days p.a., α-amylase activity decreased at a similar rate in grains from P-1a and P-1b, but in contrast, increased very rapidly in P-1c. Thus, at 28 days p.a., grains from P-1c had an α-amylase activity six times higher than that in grains from P-1a and P-1b. During the next seven days, α-amylase activity increased in grains from both P-1a and P-1b, but at a lower rate in P-1a and P-1b, and decreased in grains from P-1c. Subsequently at 35 days p.a., the highest α-amylase activity corresponded to grains from P-1c (15.43 EU per kernel) followed by the α-amylase activity in grains from P-1b (13.15 EU per kernel), with the lowest corresponding to grain from P-1a (8.38 EU per kernel). The pattern of changes in α-amylase activity observed for the highly shrivelled triticale grains was similar to that observed by Hill et al (1974) in the shrivelled triticale 6A190. The results suggest that the factors producing grain shrivelling also affect α-amylase activity in the grain.

Starch. In all three grain samples, the starch content increased from anthesis to 21 days p.a., although the rate of accumulation differed among the three samples. At 21 days p.a., grains from P-1b had the highest starch content (6.34 mg per kernel) and grains from P-1c, the lowest (3.29 mg per kernel). Thereafter the pattern of starch changes differed among the three samples. In grains from P-1a, starch content decreased between 21 to 28 days p.a., followed by a rapid increase from 28 to 35 days p.a. In contrast, the starch content of P-1b and P-1c increased until 28 days p.a then dropped during the next seven days. At 35 days p.a., grains from P-1a had the highest starch content (6.95 mg per kernel) and grains from P-1c had the lowest (3.76 mg per kernel). This trend was also observed by Noll (1977). These results indicate that the factors producing grain shrivelling also affect the accumulation of starch in the grain or, perhaps, that the extent of starch accumulation influences the degree of grain shrivelling in triticale. This is supported by results of Klassen et al (1971), who found no accumulation of starch in later developmental stages of the shrivelled triticale 6A190.

Total Soluble Sugars. The pattern of changes in total soluble sugars appeared to be more or less similar in all three samples, although rates of increase or decrease varied. The highest level of total soluble sugars in all samples occurred at 14 days p.a., followed

TABLE V
Biochemical and Chemical Composition of Categorized Triticale Grain Samples[a]

Sample No.	α-Amylase Activity[b]	Starch (%)	Total Soluble Sugars (%)	Reducing Sugars (%)
P-1a	68.3	64.3	3.2	0.12
P-1b	168.3	59.8	3.0	0.16
P-1c	1047.8	55.8	5.5	0.29
P-2a	20.5	68.0	4.2	0.09
P-2b	42.1	68.0	4.7	0.10
P-2c	485.1	61.5	7.7	0.21
P-3a	27.9	66.6	4.4	0.08
P-3b	112.2	64.8	5.0	0.09
P-3c	402.4	60.2	5.8	0.25
P-4a	42.8	68.2	4.3	0.07
P-4b	141.5	67.2	5.0	0.10
P-4c	737.2	58.8	8.5	0.24
P-5a	80.3	67.8	4.4	0.09
P-5b	171.0	64.6	4.7	0.11
P-5c	765.9	59.0	5.0	0.24
P-6a	35.4	69.6	4.3	0.04
P-6b	55.6	68.5	4.7	0.07
P-6c	367.2	59.2	7.3	0.12
P-7a	49.6	62.0	5.1	0.05
P-7b	75.3	58.7	5.6	0.08
P-7c	794.5	54.2	8.8	0.48
P-10a	92.2	63.9	4.7	0.07
P-10b	257.3	60.8	4.4	0.07
P-10c	275.9	57.7	5.2	0.08

[a] Dry weight basis.
[b] International enzyme units.

TABLE VI
Compositional Changes During Grain Development as Influenced by Degree of Shrivelling Within a Triticale Line (P-1)

Sample No.	Days After Anthesis	Moisture		α-Amylase Activity		Starch		Total Soluble Sugars		Reducing Sugars	
		(%)	H_2O Mg per kernel	EU/g[a]	EU per kernel	(%)[a]	Mg per kernel	(%)[a]	Mg per kernel	(%)[a]	Mg per kernel
P-1a	7	75.0	8.4	896.4	2.50	22.1	0.62	38.7	1.08	12.2	0.34
	14	69.2	20.5	766.1	7.00	29.8	2.72	24.0	2.19	10.5	0.96
	21	67.0	23.7	307.8	3.58	39.4	4.58	8.8	1.02	4.5	0.52
	28	66.3	18.4	315.0	2.94	37.6	3.51	4.6	0.42	2.1	0.20
	35	43.4	12.9	499.5	8.38	41.4	6.95	3.3	0.55	0.7	0.12
P-1b	7	75.4	6.0	871.0	1.69	17.6	0.34	33.7	0.66	14.2	0.28
	14	72.1	21.2	752.6	6.17	30.2	2.47	23.4	1.92	10.8	0.88
	21	68.3	30.0	283.5	3.94	45.6	6.34	7.7	1.07	3.3	0.46
	28	64.2	26.6	188.6	2.80	48.1	7.14	4.3	0.64	1.9	0.28
	35	51.6	15.5	907.2	13.15	35.0	5.07	3.4	0.49	0.8	0.12
P-1c	7	74.2	6.0	900.0	1.87	25.1	0.52	40.6	0.84	14.5	0.30
	14	73.6	16.2	709.0	4.11	24.5	1.42	18.6	1.08	5.2	0.30
	21	69.3	23.9	375.2	3.97	31.1	3.29	6.6	0.72	3.4	0.36
	28	64.3	21.4	1566.0	18.59	38.9	4.62	5.7	0.68	3.0	0.36
	35	16.7	2.2	1404.0	15.43	34.2	3.76	7.2	0.79	0.8	0.09

[a] Dry weight basis. EU = enzyme units.

by a gradual decrease. Thus, at 35 days p.a., the highest total soluble sugars content corresponded to grains from P-lc (0.79 mg per kernel), and the lowest corresponded to grains from P-1b (0.49 mg per kernel). These results did not indicate a clear relationship between total soluble sugars and grain shrivelling in triticale.

Reducing Sugars. Reducing sugars increased slightly in grains from P-1a and P-1b until the 14th day p.a., then gradually decreased to equivalent lows at 35 days p.a. In grains from P-1c, the amount of reducing sugars was fairly constant throughout grain maturation until 28 days p.a.; then it dropped dramatically the next seven days. At 35 days p.a., the amount of reducing sugars in all three samples was more or less equivalent (0.09 to 0.12 mg per kernel). We concluded, as did Noll (1977), that no real differences occurred in reducing sugars content.

In general, no evident relationship existed between α-amylase activity and starch content. The high α-amylase activities in mature grain and in its flour (Tables III and V), regardless of the extent of grain shrivelling, indicate that total soluble sugars and reducing sugars should have been higher than observed. Perhaps the grain utilizes free sugars (both total and reducing) for energy, for biosynthesis of starch or other carbohydrates, or both. The changes in total soluble and reducing sugars observed during grain development (Table VI) support the biosynthesis hypothesis. The observed relationships between grain shrivelling and starch content (Tables V and VI) are interpreted as starch accumulation being influenced by the factors that cause grain shrivelling. Because Jenner and Rathjen (1972a, 1972b) observed that an unidentified mechanism limits the accumulation of sucrose in wheat, this can logically be extended to triticale as a part of the maturation or senescence mechanisms. Based on the high frequency of aberrant endosperm nuclei observed in highly shrivelled triticales (Bennett 1977, Kaltsikes et al 1975), triticale developing with limited endosperm cell numbers (the endosperm sink) should halt the flow of nutrients earlier than in plump grains and, hence, reach physiological maturity earlier. That was observed in the pronounced drop in moisture content at 35 days p.a. in grains from P-lc, whereas in grains from P-1a and P-1b, water was lost at a much slower rate (Table VI). P-1a and P-lb would be expected to reach physiological maturity several days later and would accumulate more starch during the 28 to 35 days p.a. than would the more shrivelled grain sample P-1c. These observations are supported by Hill et al (1974) who found that a shrivelled triticale transports less labeled sucrose to the head than a plump one; by Agrawal (1977) who observed that starch content reaches its maximum at 35 days p.a. in a shrivelled triticale and at 42 days p.a. in a plump one; and by Srivastava (1978) who suggested that declining starch concentrations 30 days p.a. could be related to decreased capacity of shrivelled triticale grains to accept metabolized nutrients.

Morphological observations of mature and developing grains from these same triticale grain classes further support these biochemical findings as well as unify the concepts of seed shrivelling (Peña et al 1982).

ACKNOWLEDGMENTS

R. J. Peña gratefully acknowledges the scholarship for graduate studies provided by the National Council of Science and Technology (CONACYT) of Mexico and the CIMMYT wheat staff who provided materials and information.

LITERATURE CITED

AGRAWAL, P. K. 1977. Changes in amylase, starch and reducing sugars during grain development in triticale and their relation to grain shrivelling. Cereal Res. Commun. 5:225.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. 1965. Official methods of analysis. 10th ed. The Association, Washington, DC.

BARNES, W. C., and BLAKENEY, A. B. 1974. Determination of cereal α-amylase using a commercially available dye-labelled substrate. Staerke 26:193.

BENNETT, M. D. 1974. Meiotic, gametophytic, and early endosperm development in triticale. Page 137 in: Triticale: Proc. Int. Symp., El Batan, Mexico, 1–3 October 1973, Int. Develop. Res. Centre Monogr. IDRC-024e.

BENNETT, M. D. 1977. Heterochromatin, aberrant endosperm nuclei and grain shrivelling in wheat-rye genotypes. Heredity 39:411.

BENNETT, M. D., SMITH, J. B., and BARCLAY, I. R. 1975. Early seed development in the triticale. Philos. Trans. R. Soc. London B272 916:199.

CHOJNACKI, G., BRYKCZYINSKI, J., and TYMIENIECKA, E. 1976. Preliminary information on sprouting in triticale. Cereal Res. Commun. 4:111.

DEDIO, W., SIMMONDS, D. H., HILL, R. D., and SHEALY, H. 1975. Distribution of α-amylase in the triticale kernel during development. Can. J. Plant Sci. 55:29.

GREENWOOD, C. T., and MILNE, E. A. 1968. Studies on starch-degrading enzymes. Part VII. Properties and action-pattern of the α-amylases from barley, oats, rye and wheat. Staerke 20:101.

HILL, R. D., KLASSEN, A. J., and DEDIO, W. 1974. Metabolic factors influencing kernel development in triticale. Page 149 in: Triticale: Proc. Int. Symp., El Batan, Mexico, 1–3 October 1973. Int. Develop. Res. Centre Monogr. IDRC-024e.

JENNER, C. F., and RATHJEN, A. J. 1972a. Factors limiting the supply of sucrose to the developing wheat grain. Ann. Bot., London 36:729.

JENNER, C. F., and RATHJEN, A. J. 1972b. Limitations to the accumulation of starch in the developing wheat grain. Ann Bot., London 36:743.

KALTSIKES, P. J., and ROUPAKIAS, D. G. 1975. Endosperm abnormalities in Triticum-Secale combinations. II. Addition and substitution lines. Can. J. Bot. 53:2068.

KALTSIKES, P. J., ROUPAKIAS, D. G., and THOMAS, J. B. 1975. Endosperm abnormalities in Triticum-Secale combinations. I. X-Triticosecale and its parental species. Can. J. Bot. 53:2050.

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

LORENZ, K., and WELSH, J. R. 1976. Alpha-amylase and protease activity of maturing triticale and its parental species. Lebensm. Wiss. Technol. 9:7.

McCREADY, R. M., GUGGOLZ, J., SILVIERA, V. V., and OWENS, H. S. 1950. Determination of starch and amylase in vegetables. Anal. Chem. 2:1156.

NELSON, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153:375.

NOLL, J. S. 1977. Changes in the proteins and carbohydrates of developing cereal grains and their relationship to kernel shrivelling in triticale. Diss. Abstr. Int. B 38(2):434.

PEÑA, R. J., NAGARAJAN, P., and BATES, L. S. 1982. Grain shrivelling in secondary hexaploid triticale. II. Morphology of mature and developing grains related to grain shrivelling. Cereal Chem. 59:459.

RAO, V. R., MEHTA, S. L., and JOSHI, M. G. 1976. Peroxidase and amylase activity in developing grains of triticale wheat and rye. Phytochemistry 15:893.

ROBYT, J. F., and WHELAN, W. J. 1968. The α-amylases. Page 431 in: Starch and Its Derivatives. J. A. Radeley, ed. Chapman and Hall, London.

SALMINEN, S. O., and HILL, R. D. 1978. Rate and duration of dry matter accumulation in relation to kernel shrivelling in triticale. Can. J. Plant Sci. 58:13.

SIMMONDS, D. H. 1974. The structure of the developing and mature triticale kernel. Page 105 in: Triticale: First Man-Made Cereal. C. C. Tsen, ed. Am. Assoc. Cereal Chem., St. Paul, MN.

SIMMONDS, D. H., and CAMPBELL, W. P. 1976. Morphology and chemistry of the rye grain. Page 71 in: Rye-Production, Chemistry and Technology. W. Bushuk, ed. Am. Assoc. Cereal Chem., St. Paul, MN.

SINGH, B., PATEL, J. A., and SAPRA, V. T. 1978. Amylase activity in triticale, X Triticosecale Wittmack. Euphytica 27:19.

SRIVASTAVA, A. K. 1978. Physiology of kernel shrivelling and its reduction in triticale. Plant and Cell Physiology 19:337.

STRUMEYER, D. H. 1967. A modified starch for use in amylase assays. Anal. Biochem. 19:61.

[Received July 16, 1981. Accepted March 11, 1982]