Changes in the Alpha-Amylase and Protease Activities of Four Secondary Hexaploid Triticales During Kernel Development[1]

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ABSTRACT

The α-amylase, exoprotease, and endoprotease activities of four secondary hexaploid triticales and one hard red spring wheat were followed from 21 days post-anthesis to full maturity during the 1983 and 1984 growing seasons. α-Amylase activity of the triticales generally increased during kernel development, while exo- and endoprotease activities generally decreased. At full maturity, all triticales were higher in whole grain α-amylase and protease activities than Marquis wheat checks grown in the same year. Of the three enzyme systems examined, differences in α-amylase activity due to cultivar and year were most apparent.

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Studies show that increasing levels of α-amylase and protease activities during germination or field sprouting may lead to degradation of starch and storage proteins in the endosperm, which in turn may have a deleterious effect on breadmaking quality (D'Appolonia 1983, Lorenz et al 1983, Lukow and Bushuk 1984). However, little attention has been given to the variation in levels of such enzymes during kernel development, and the possible influence that such variations might have in altering the functional properties of endosperm components. The present study was initiated to determine the levels of α-amylase and protease activities in the maturing grain of four secondary hexaploid triticales (hybrids derived from triticale × bread wheat crosses) of diverse baking quality, and the effects which these enzymes might have on endosperm starch and storage (gluten) proteins.

Both exoproteolytic and endoproteolytic enzymes exist in cereals, but the findings of Preston et al (1978) indicate that an increase in endoprotease activity is required for extensive storage protein hydrolysis during germination. Accordingly, both exoprotease (hemoglobinase) and endoprotease (azocaseinase) activities were assayed. To date, there is no report in the literature on the changes in endoprotease activity of developing triticale kernels.

MATERIALS AND METHODS

Triticale and Wheat Samples

Four secondary hexaploid triticales (4T, 11T, Impala, and Carman) and one hard red spring wheat (cultivar Marquis) were included in the study. The chromosome composition of the triticale samples is described elsewhere (Macri et al 1986). All lines were grown in dryland field plots at the University of Manitoba during the 1983 and 1984 growing seasons. Heads were tagged on the day of anthesis, and samples were collected at 21, 28, 32, 36, 40, and 44 days post-anthesis (DPA). The kernels from five heads were removed immediately for fresh weight and moisture determinations. The remaining 50–70 heads were frozen (−20° C) and freeze-dried. Dried heads were threshed on a single-head roller thresher, and kernels were separated with an Erikson column blower. For each sample, 50 g of seed was ground in a Udy cyclone mill (1.0-mm screen) for whole meal analyses. All assays were conducted in duplicate.

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Moisture and Protein Content

Moisture was determined by drying to constant weight in a 110° C oven. Protein (N × 5.7) was determined by the macroKjeldahl method of Williams (1973) using a TiO_2 catalyst.

α-Amylase Activity

The Perkin-Elmer model 191 Grain Amylase Analyzer (GAA) was operated according to the method of Kruger and Tipples (1981). The substrate, extracts, standard enzymes, and all dilutions described below were prepared with 0.05*M* sodium acetate buffer (pH 5.5) containing 1 m*M* $CaCl_2$.

The β-limit dextrin substrate was prepared according to the method of Kruger (1972) using amioca pearl starch (American Maize Products). Before freeze-drying, the dextrin solution was boiled for 5 min to inactivate the sweet potato β-amylase (Sigma Chemical Co.). Working substrate (0.5% dextrin) was prepared fresh daily according to the method of Kruger and Tipples (1981) and stored overnight at 4° C before use.

Whole meal (1.0 g) was extracted in 5.0 ml of acetate buffer at room temperature for 1 hr on a variable speed rotator at 9 rpm. Suspensions were filtered (Whatman GF glass fiber paper), and filtrates were stored overnight at 4° C. Because of day-to-day variations in the nephelos reading of the substrate, the GAA was calibrated with a set of dilutions of fungal α-amylase (*Aspergillus oryzae*, Miles Scientific). α-Amylase activity was measured as the change in turbidity of the digest with time and is reported in arbitrary GAA units per gram of dry meal.

Exoprotease (Hemoglobinase) Activity

Exoprotease activity was determined by a modification of the method of Bushuk et al (1971). Whole meal (0.1 g) was suspended in 5.0 ml of 1% hemoglobin (bovine type II, Sigma Chemical Co.) dissolved in 0.2*M* sodium acetate buffer (pH 4.5), and incubated for 2 hr at 37° C with mixing at half-hour intervals. The reaction was stopped with 5.0 ml of cold 10% trichloroacetic acid (TCA) and clarified by centrifugation (27,000 × *g* for 10 min). Aliquots of supernatant were made up to 2.0 ml with acetate buffer and analyzed for TCA-soluble nitrogen according to the method of Moore and Stein (1954). Blanks were determined by incubating whole meal with TCA, adding substrate after 2 hr, and proceeding as above. Glutamate (0–60 μg/2 ml buffer) was used to obtain a standard curve.

Endoprotease (Azocaseinase) Activity

Endoprotease activity was determined by the method of Preston et al (1978) with the following modifications. The azocasein substrate was dialyzed overnight at 4° C against 0.05*M* McIlvaines buffer (pH 6.0) to reduce the substrate blank. Whole meal (1.2 g) was extracted in 7.0 ml of 0.05*M* sodium acetate buffer (pH 5.5) for 1 hr at 4° C on a variable speed rotator (9 rpm). Suspensions were filtered (Whatman GF paper), and filtrates were immediately incubated with dialyzed substrate for 4 hr at 40° C with mixing at l-hr intervals. After termination of the reaction, precipitated protein was removed by centrifugation (24,000 × *g* for 15 min).

RESULTS AND DISCUSSION

Moisture contents, fresh weights, and protein contents of the developing kernels are shown in Figure 1. Growing conditions were unusually warm and dry in 1983, which explains the rapid loss of moisture and higher protein content of the 1983 grain samples. No attempt was made to separate total nitrogen into protein-incorporated versus nonprotein nitrogen, although it was noted that the amount of TCA-soluble nitrogen in all cultivars (estimated from exoprotease blanks) decreased steadily with maturation until approximately 32 DPA and remained at relatively constant levels thereafter. The accumulation of dry matter in the developing kernels (data not shown) closely paralleled total nitrogen increases and peaked between 28 and 32 DPA in both 1983 and 1984.

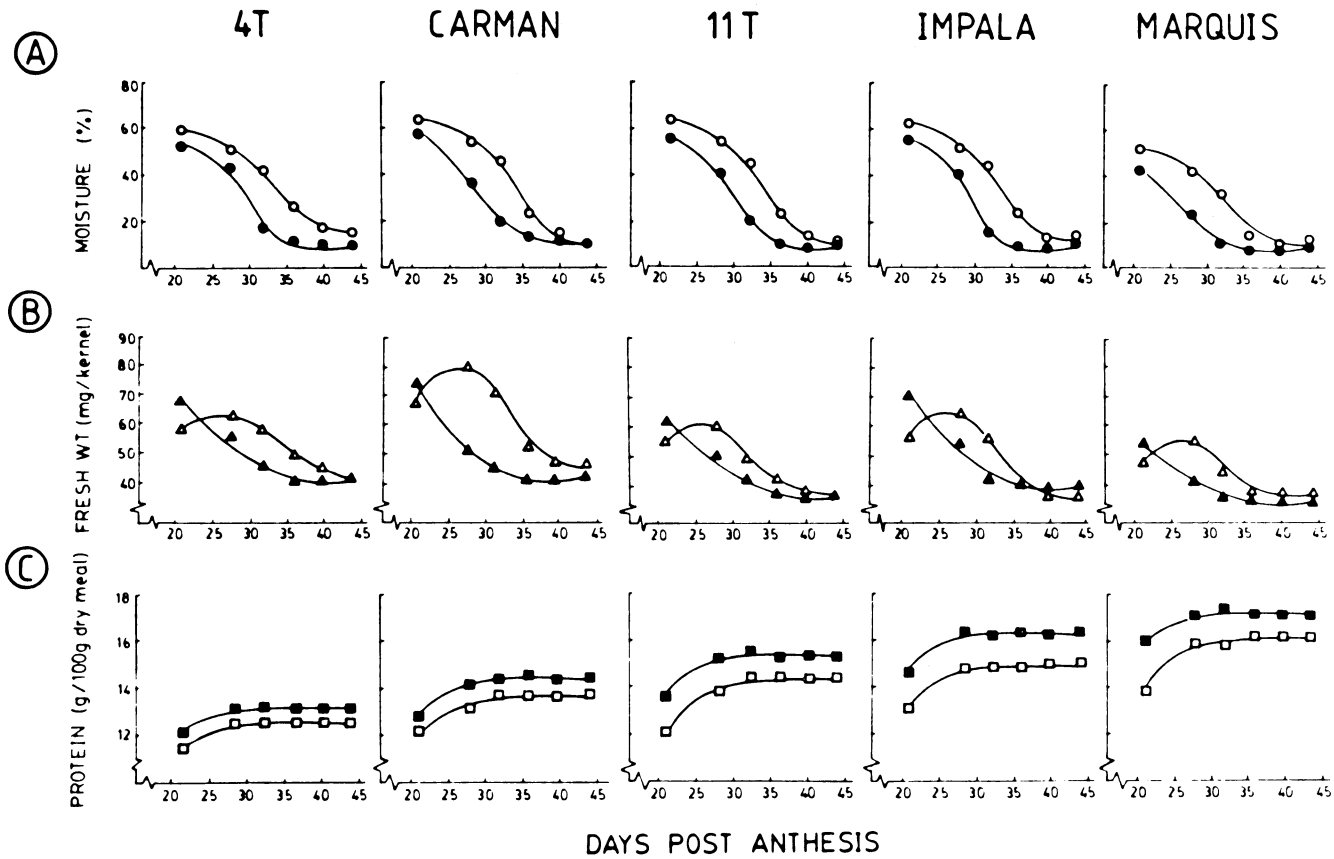

Fig. 1. Changes in (**A**) moisture content, (**B**) fresh weight, and (**C**) protein content (N × 5.7) during kernel development in the 1983 (●, ▲, ■) and 1984 (O, △, □) growing seasons.

α-Amylase Activity

α-Amylase activity in the triticales generally increased during grain maturation, whereas activity in the Marquis wheat checks remained very low in the later stages of kernel development (Fig. 2). At harvest ripeness (44 DPA), α-amylase activity was 10–200 times higher in the triticale samples than in the Marquis wheat checks. Levels of α-amylase activity in the developing Carman and 11T kernels were relatively stable and similar in both years. In contrast, the α-amylase activity in the 1984 4T and Impala samples increased sharply after 32 DPA, peaked at 36 DPA, and then decreased in the final stages of grain maturation. Similar increases in α-amylase activity in other triticale cultivars have been reported by Dedio et al (1975), Lorenz and Welsh (1976), and Peña and Bates (1982).

The sudden increase in α-amylase activity in the 1984 4T and Impala samples occurred in the absence of rain. It should be noted, however, that the moisture content of the 4T and Impala kernels at 32 DPA was approximately 30% higher in 1984 than in 1983 (Fig. 1A). Gale et al (1983) similarly reported that the production of α-amylases in maturing wheats just before harvest ripeness can be enhanced by slow drying conditions.

Recent findings of Weselake et al (1985) indicate that endogenous α-amylase inhibitors that have been purified from barley are also present in wheat, rye, and triticale. Warchalewski (1977) suggested that the dissociation of α-amylase-inhibitor complexes already present in mature grain might supplement de novo synthesis of this enzyme in the early stages of germination. Premature dissociation of such complexes might similarly explain the increase in α-amylase activity in the developing kernels of triticale cultivars such as 4T and Impala.

Exoprotease (Hemoglobinase) Activity

A preliminary study in 1983 showed that the optimum pH for hemoglobinase activity of 4T (28 DPA) and 11T (36 DPA) whole meal suspensions was 4.5. This agrees closely with the pH optimum of 4.45 reported by Madl and Tsen (1973) for hemoglobinase activity in triticale and wheat flour suspensions.

Exoprotease activity in the triticale and Marquis wheat samples decreased from 21 to 32 DPA and then remained at relatively constant levels (Fig. 3). This result is in general agreement with the findings of Lorenz and Welsh (1976), who reported that hemoglobinase activity in various triticales and durum wheats decreased from initial kernel development to full maturity. All triticales had higher levels of exoprotease activity than Marquis wheat checks grown in the same year throughout kernel development, and exoprotease activity at 44 DPA was 1.2–2.0 times higher in the triticales than in the wheat samples. For any given cultivar, levels of exoprotease activity in the maturing grain was relatively stable from year to year.

Endoprotease (Azocaseinase) Activity

Endoprotease activity in the triticale and wheat samples decreased throughout kernel development (Fig. 4). At full maturity, endoprotease activity was 1.5–2.3 times higher in the triticale samples than in Marquis wheat checks grown in the same year. Endoprotease activity in both the triticale and wheat samples was higher in 1984 than in 1983, suggesting that endoprotease activity was influenced to a greater extent by environment than exoprotease activity. It is interesting to note that levels of endoprotease activity in the 1983 triticale samples were comparable to levels in the 1984 Marquis wheat check.

Whereas the four lines of secondary triticale examined had higher levels of α-amylase and protease activity than Marquis wheat checks, there are three observations that indicate in situ degradation of seed reserves during kernel development was limited. With the exception of 4T, levels of damaged starch were lower in the triticale flours than in the Marquis flours, and levels of damaged starch were relatively constant from year to year for any given cultivar despite yearly fluctuations in α-amylase activity (Macri et al 1986). Secondly, levels of TCA-soluble nitrogen in the maturing kernels remained relatively constant after 32 DPA, indicating that there was no rapid increase in free amino acids as a consequence of exoproteolytic degradation of storage proteins.

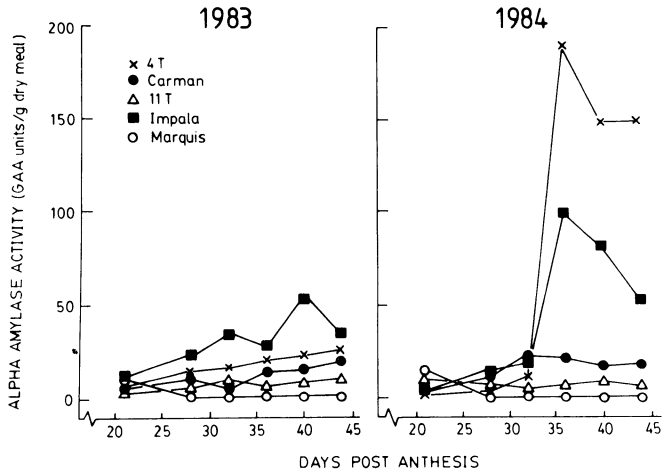

Fig. 2. Changes in α-amylase activity during kernel development.

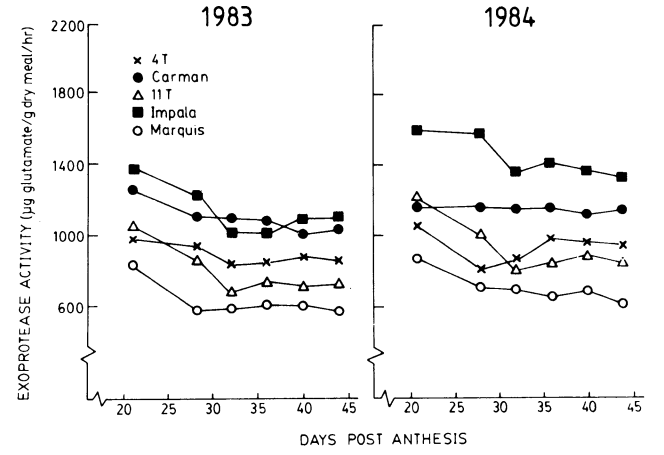

Fig. 3. Changes in exoprotease activity during kernel development.

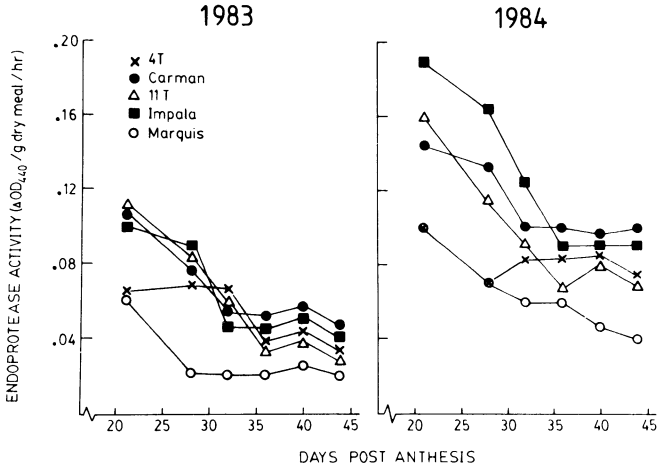

Fig. 4. Changes in endoprotease activity during kernel development.

Thirdly, α-amylase, exoprotease, and endoprotease activities were noticeably higher in whole-meal samples than in the milled flours (Macri et al 1986), suggesting that a large portion of the enzyme activity in the whole grain was confined to the outer kernel layers (e.g., the aleurone layer) and was not released into the endosperm during kernel development. Despite the higher α-amylase (Fig. 2) and protease (Figs. 3 and 4) activities in the developing Impala kernels, flours milled from mature grain of this cultivar still produced breads with satisfactory loaf volumes and crumb textures (Macri et al 1986).

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