CHANGES IN THE CATALASES OF WHEAT DURING KERNEL GROWTH AND MATURATION[1]

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ABSTRACT

Changes in catalase activity have been determined for two hard red spring and two durum wheat cultivars during growth and maturation. Activity increased during early kernel growth and decreased as the kernel ripened. Maximal activity occurred at a later stage of development in durum than in hard red spring wheats. The majority of the catalase activity was present in the endosperm during development of the kernel. The rise and fall in activity of this tissue accounted primarily for changes seen in the whole kernel. Polyacrylamide slab electrophoresis indicated that different anatomical parts of the immature wheat kernel contained electrophoretically different catalase isozymes. Upon germination of Manitou wheat, catalase activity increased to twice the maximal amount present in the immature kernel. No new isozymes appeared during germination of this cultivar, but an additional component was observed in the durum wheat, Hercules.

Catalase is a hemoprotein which catalyzes the breakdown of hydrogen peroxide into oxygen and water. One of its roles in plants is believed to be the prevention of harmful hydrogen peroxide accumulation (1). The enzyme has been proposed to be involved in oxidative reactions during breadmaking (2,3) and the bleaching of pigments (4). These involvements, however, remain to be established.

The variable and low levels of catalase in the resting state of wheat kernels depend on cultivar and geographical location of growth (5,6). Upon germination, activity increases two- to fivefold over a period of 3 to 5 days (7–9). During growth and maturation, changes in catalase levels are much less certain. Bach *et al.* (7) showed that the enzyme increased during growth and decreased irregularly as the kernel matured. A similar result was found by Tetova (10), in which catalase activity decreased with decreasing moisture content as the kernel ripened. On the other hand, catalase activity has been reported to increase (11) and remain constant (9) during ripening.

The present study was undertaken, therefore, to gain a better understanding of the catalase system present in wheats grown in North America. Two hard red spring and two durum wheats were analyzed for total level of catalase during kernel development, and the anatomical distribution of activity was determined for Manitou wheat. Levels present in germinating kernels were determined for comparative purposes. In addition, polyacrylamide slab electrophoresis was used to gain a further insight into the isozymic nature of wheat catalase.

MATERIALS AND METHODS

The varieties used in the study were: Prairie Pride and Manitou (hard red spring), and Stewart 63 and Hercules (durum).

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For studies on developing kernels, wheats were planted on May 10, 1972, at the University of Manitoba experimental plots, Winnipeg, Manitoba. Following flowering, the cultivars were sampled at 2- to 3-day intervals. The excised heads were stored in a deep freeze.

Germination was performed by placing 100 kernels of wheat in a 13 × 13.5-cm covered sample dish on top of two 13 × 13.5-cm sheets of germination paper moistened with 16 ml of water. The samples were then placed in a dark moisture cabinet at 23°C and removed after germination times ranging from 1 to 5 days. The germinated samples were stored frozen prior to analysis.

Extraction of Wheat Kernels

Ten kernels were ground in a mortar and pestle with 4 ml of 12.5% sucrose. The suspension was centrifuged at 25,000 × *g* for 10 min, and the supernatant was assayed.

Catalase Activity

Enzyme activity was determined by the method of Sinha (12), which was semiautomated on the Technicon AutoAnalyzer (13). The observable rate constant (k) was used as a measure of enzyme activity. A unit of catalase was defined as having a k of 1×10^{-4}, sec^{-1} at 25°C.

Polyacrylamide Slab-Electrophoresis and Detection of Isozymes

Electrophoresis was performed at pH 9.0 (14) using an Ortec Model 4200 slab-electrophoresis system. Isozymes were detected by the method of Woodbury *et al.* (15). The gel was incubated for 15–20 min in 0.003% hydrogen peroxide prior to adding 1% ferric chloride and potassium ferricyanide. Catalase isozymes appeared as yellow bands against a dark green field.

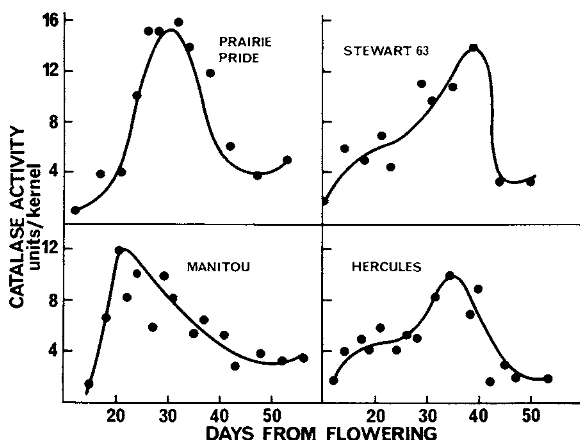

Fig. 1. Changes in total catalase activity of the hard red spring wheats Prairie Pride and Manitou and the durum wheats, Stewart 63 and Hercules, during kernel growth and maturation.

Dissection Techniques

Dissections were performed as described previously (16). The tissues separated were: pericarp, green layer, and testa, aleurone, endosperm, scutellum, and embryo.

RESULTS AND DISCUSSION

Catalase Activity in Growing and Maturing Wheat Kernels

The changes in catalase activity during growth and maturation of the wheats are shown in Fig. 1. Activity increased in each cultivar during early developmental stages and then decreased with maturation. This confirms the previous findings of Bach *et al.* (7) and Tetova (10). The maximal activity of the hard red spring wheats appeared at 20–30 days after flowering. In the case of the durum wheats, the peak activity occurred 35–40 days after flowering. Although maximal activities varied with the different cultivars, differences in activities at maturity were minimal. It is apparent, however, that the level of catalase activity, particularly in the case of durum wheats, would be quite dependent on the date on which the wheat was harvested.

To determine the anatomical location of catalase, kernels of Manitou wheat at 20, 31, and 40 days after flowering were dissected and analyzed quantitatively. A typical anatomical distribution of catalase activity is shown in Fig. 2. Endosperm tissue contained the bulk of the catalase activity in the developing wheat kernel. This activity decreased with maturation to a very low level and undoubtedly accounted for the major decreases in total activity of Manitou wheat shown in Fig. 1. In this regard, the catalase enzymes behave similarly to the polyphenol oxidase system (17) but unlike the peroxidase system (16), which is mainly

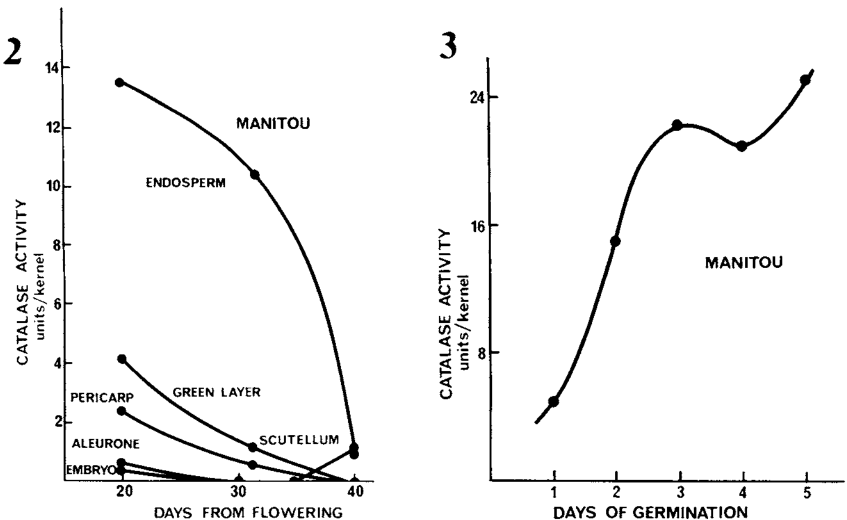

Fig. 2. Anatomical distribution of catalase activity during growth and maturation of Manitou wheat kernels. Fig. 3. Change in catalase activity of Manitou wheat upon germination.

concentrated in the pericarp of the immature kernel. Catalase activity was found in other tissues of the developing wheat kernel 20 days after flowering, except for the scutellum, but the levels were much smaller. These activities decreased with kernel maturation. The scutellum was the only tissue in which catalase activity increased during the final maturation stages.

Catalase Activity of Germinating Wheat Kernels

The hard red spring wheat, Manitou, was used to compare the maximum level reached in the immature kernel with that obtainable upon germination. As

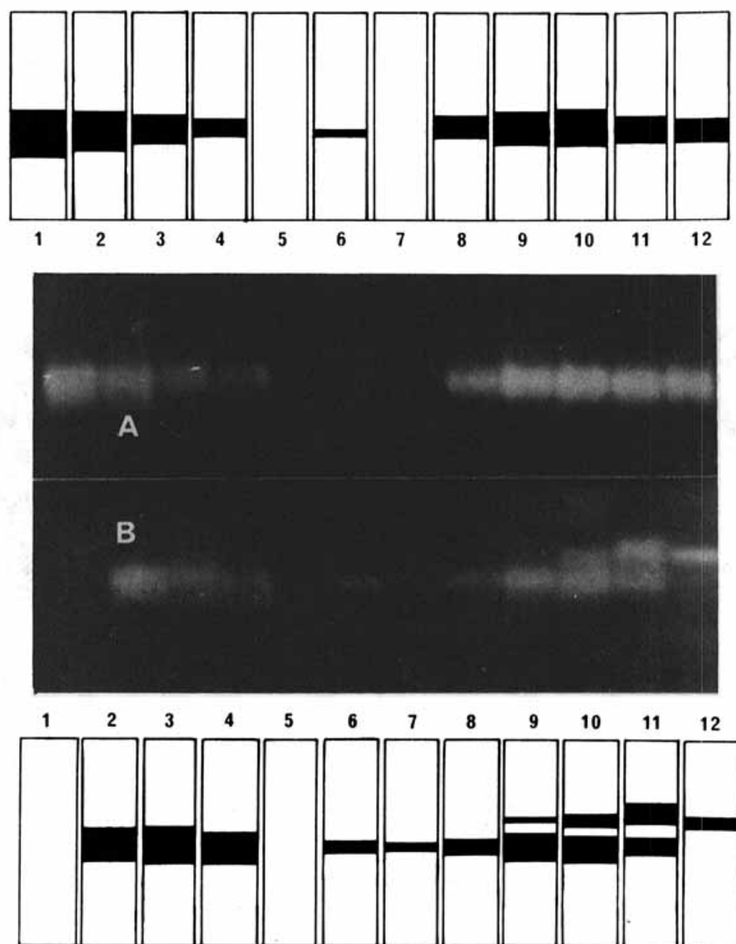

Fig. 4. Changes in catalase isozymes during growth, maturation, and germination of wheat kernels. A) Manitou, B) Hercules. Sample slots 1–12 for each variety contained, in order, the following: immature kernels at 17, 27, 29, 35, 40, and 45 days after flowering, and kernels germinated for 1, 2, 3, 4, 5, and 6 days. Samples were applied to wells at the top of the gels and isozymes migrated downward toward the anode.

shown in Fig. 3, catalase activity rose from 4.9 to 24.9 units/kernel from day 1 to day 5 of germination. This fivefold increase in activity is in agreement with the findings of previous workers (7,8). The reported (10) gradual decrease in activity past day 3 was not observed. The level reached after day 5 was twice the maximum level obtained during kernel development. This suggests the possibility that part of the increased activity during germination occurs by *de novo* synthesis.

Catalase Isozymes in Developing and Germinating Wheat Kernels

Extracts of developing and germinating kernels of Manitou and Hercules were examined for catalase isozymes by polyacrylamide-slab electrophoresis. Six stages in the development of the wheat kernel and 6 days of germination were analyzed simultaneously on one slab. The results are shown in Fig. 4. One major band was observed in Manitou wheat. This band decreased as the kernel matured, but reappeared and increased during germination. Similar behavior was observed with Hercules, except that as germination proceeded, an electrophoretically less mobile band appeared synchronously with the disappearance of the major catalase band. This indicates that the number of isozymes formed is dependent upon the genetic background of the wheat.

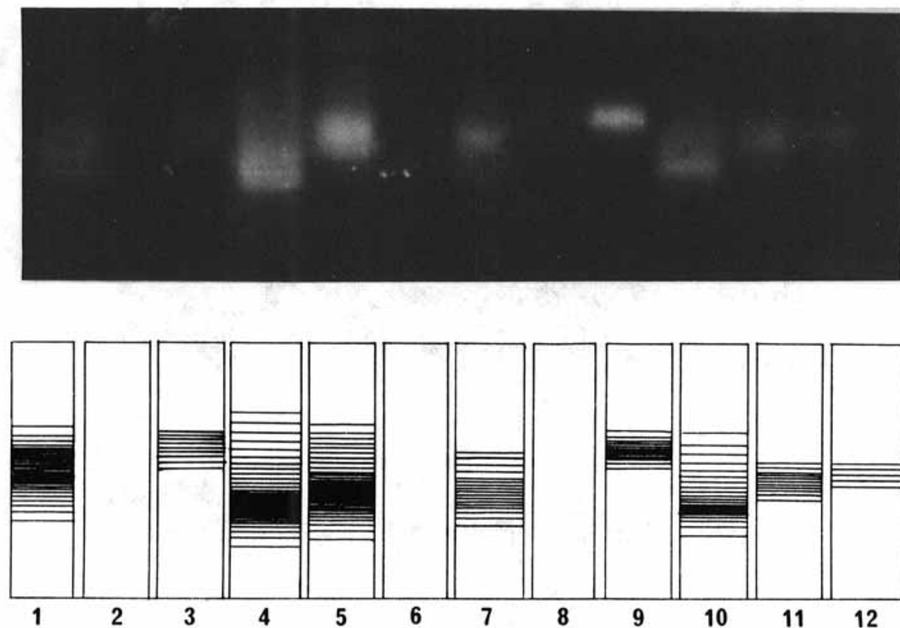

Fig. 5. Catalase isozymes in anatomical parts of Manitou (slots 1–6) and Hercules kernels (slots 7–12) at 25 days after flowering. Slots 1, 7—pericarp; slots 2, 8—embryo; slots 3, 9—scutellum; slots 4, 10—endosperm; slots 5, 11—green layer; and slots 6, 12—aleurone. Samples were applied to wells at the top of the gels and isozymes migrated downward toward the anode.

Electrophoretically identical bands have also been observed in sound and germinated hard red spring wheat by Macko *et al.* (18). Singh and Singh (19), however, have reported that different isozymic forms of catalase arise during germination.

With the oxidizing enzyme systems, polyphenol oxidase and peroxidase, different isozymes exist in different anatomical parts of the wheat kernel (16,17). To see if this was the case for wheat catalase, Hercules and Manitou were dissected at 25 days after flowering and extracts electrophoresed. It was found (Fig. 5) that electrophoretically different catalases were present in the different tissues of the kernel. The mobility of the isozymes in the different tissues of Hercules and Manitou was very similar. It was also evident that the diffuse bands observable in whole kernel extracts (Fig. 4) were actually composed of the electrophoretically similar bands present in the different tissues. The most electrophoretically mobile and intense bands were in the endosperm of each cultivar. Similar but decreased isozymic mobilities were present in the green layer, aleurone, and pericarp. The electrophoretically least mobile catalase was present in the pericarp. At 25 days after flowering, the catalase present in the embryo was extremely low and, consequently, no catalase band could be detected in this tissue.

Extracts of 12 additional hard red spring wheats of varying breadmaking quality and 12 durum wheat cultivars with differences in semolina color were examined for catalase isozymes to determine if intervarietal differences could be related to quality. No significant intervarietal differences were found, except for two durum varieties which contained a slightly more mobile diffuse isozyme. The effect of catalase upon wheat quality, however, may take place at an earlier stage of kernel maturity when the level of catalase activity is much higher. Most of this activity resides in the endosperm and it has been suggested that catalase may utilize the amino residues of proteins as hydrogen donors, thus affecting protein polymerization (3). More research will be needed, therefore, to clearly establish relations that exist between the mode of insolubilization of storage proteins and catalase activity in the developing kernel.

Acknowledgments

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