

# CHANGES IN BARLEY PEROXIDASE ACTIVITY DURING KERNEL DEVELOPMENT<sup>1</sup>

D. E. LaBERGE and J. E. KRUGER

## ABSTRACT

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Peroxidase activity in whole kernels and discrete kernel tissues from two barley cultivars (*Hordeum vulgare* L. and *Hordeum distichum* L.) was determined at various stages of kernel development. Differences in peroxidase activity were noted in similar tissues dissected from the two cultivars at comparable stages of development. Much of the peroxidase activity was associated with endosperm tissue in both cultivars, but the

amount varied at different stages during ripening. Only low levels of peroxidase were found in the hulls, pericarp, and green layer from each variety. Higher levels of peroxidase activity were detected in aleurone tissue and in the embryo and scutellum of Bonanza barley than were detected in similar tissues from Centennial barley. Barley peroxidase activities were stable to heat, as less than 50% of the total activity was lost after 15 min at 70°C.

A previous study (1) demonstrated the presence of 14 peroxidase isozymes in mature barley kernels. A further study (2) demonstrated the alteration of barley peroxidase isozymic patterns during maturation, and the anatomical distribution of these isozymes in the different tissues of the mature kernel. The present study was undertaken to determine the quantitative changes of peroxidase activity that occur in different tissues of barley kernels during maturation.

## MATERIALS AND METHODS

The barley samples were grown under field conditions. A complete record of the dates of seeding, anthesis, threshing, and climatic data relating to the samples has been published (3). The barleys studied included the six-rowed malting cultivar, Bonanza (*Hordeum vulgare* L.), and the two-rowed malting cultivars, Centennial and Betzes (*Hordeum distichum* L.).

### Dissection Studies

Kernels used for dissection were selected from ears of barley tagged during anthesis and maintained in a freezer following harvest at each stage of ripening. Five kernels at each stage of development were dissected. The tissues in order of removal during dissection included husk, pericarp, green layer, embryo, scutellum, aleurone, and endosperm. The dissection technique (4) and a description of the tissue fractions (2) were described previously.

The different dissected tissue fractions were placed in 2 ml of 12.5% sucrose. After grinding with a mortar and pestle, the suspensions were centrifuged at 25,000 × g for 10 min. The clear supernatant solutions were used for quantitative assays of peroxidase activity.

### Determination of Peroxidase Activity

Peroxidase activity was determined using hydrogen peroxide as the substrate

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and *o*-dianisidine as the hydrogen donor (5). A complete description of the assay was published previously (6). One unit of peroxidase activity was defined as that amount of enzyme that consumed 1  $\mu$ mol of peroxide per min at 25°C.

#### Heat Stability of Barley Peroxidase

Kernels of mature Bonanza and Betzes barley (25 g) were ground in a Krups 75 coffee mill for 1 min. Ground samples (20 g) were extracted with 40 ml of 12.5% sucrose by gently shaking the suspension on a shaking apparatus for 30 min. Following centrifugation, aliquots were heated for 15 min at 30°, 40°, 50°, 60°, 70°, and 80°C, quickly cooled in an ice bath, and assayed for peroxidase activity.

### RESULTS

Deposition of kernel dry matter is a useful indication of kernel maturity (3). Changes in kernel dry matter for the barley cultivars are shown in Fig. 1. Kernels of the six-rowed cultivar, Bonanza, developed more quickly than kernels of the two-rowed cultivar, Centennial. The major dry weight deposition for kernels of

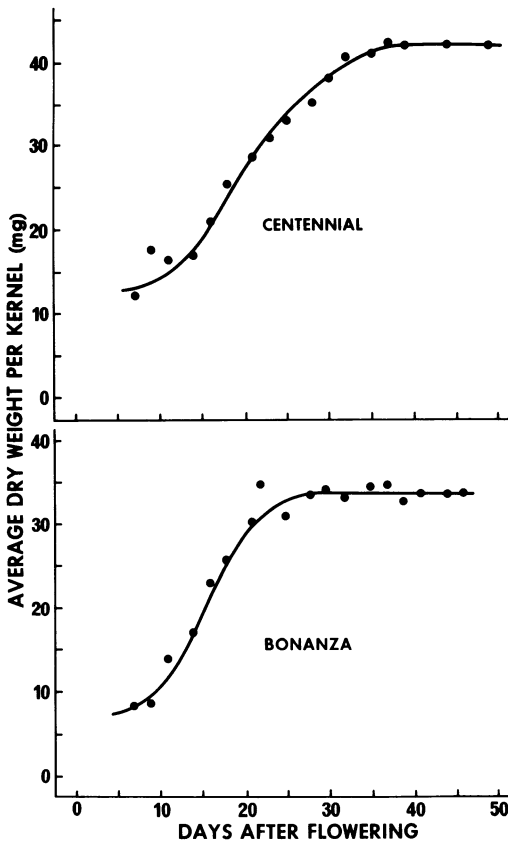


Fig. 1. Increase in dry matter during the development of barley kernels.

Bonanza barley occurred between 10 and 25 days post-anthesis. An additional 10 days were required for full maturity of Centennial barley.

Changes in peroxidase activity in whole kernels and in dissected tissues are shown in Fig. 2. Total peroxidase activity of each cultivar increased during the early stages of kernel development, then decreased and, finally, increased again as the kernels became fully mature. These changes occurred at different stages of kernel development for the two barley cultivars.

Changes in peroxidase activity in whole kernels of Centennial barley are largely due to changes in activity that occur in the endosperm (Fig. 2). Low levels of peroxidase activity were observed in other tissues except for the aleurone, where peroxidase activity increased as the kernel became fully mature. On the other hand, peroxidase activity of tissues from Bonanza barley was more variable. Peroxidase activity was observed to rise and fall in several tissues including the aleurone, embryo, scutellum, and endosperm between 12 and 20 days post-anthesis. There was a large increase in peroxidase activity of

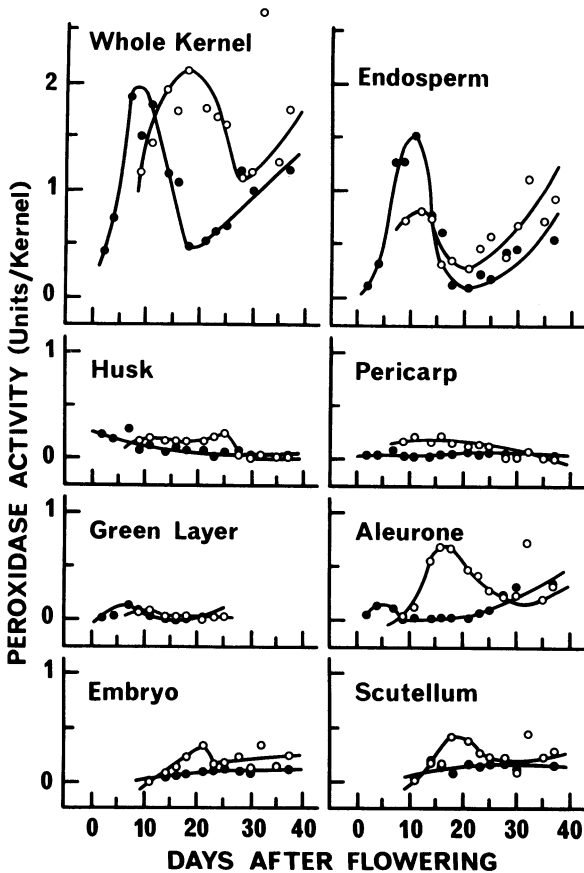


Fig. 2. Changes in total peroxidase activity of whole kernels and kernel tissue fractions during kernel development: o—o Bonanza barley, •—• Centennial barley.

endosperm tissue as Bonanza kernels became fully ripened.

Changes in the distribution of peroxidase activity in the various tissues at different stages of kernel development are shown in Fig. 3. Immediately following anthesis, most of the activity of Centennial barley was associated with the husk. However, as the kernels developed, the hulls contributed a decreasing proportion of peroxidase activity. A high percentage of total peroxidase activity was localized in endosperm tissue during early stages of development. This percentage then decreased until 20 days after floral anthesis, when the amount increased rapidly until full maturity. Differences in anatomical location of peroxidase activity between the two cultivars were also apparent, particularly in the pericarp, aleurone, embryo, and scutellum. For example, peroxidase activity associated with the aleurone of Bonanza barley decreased from 40% at 15 days following floral anthesis to 17% at maturity. In Centennial barley, the low

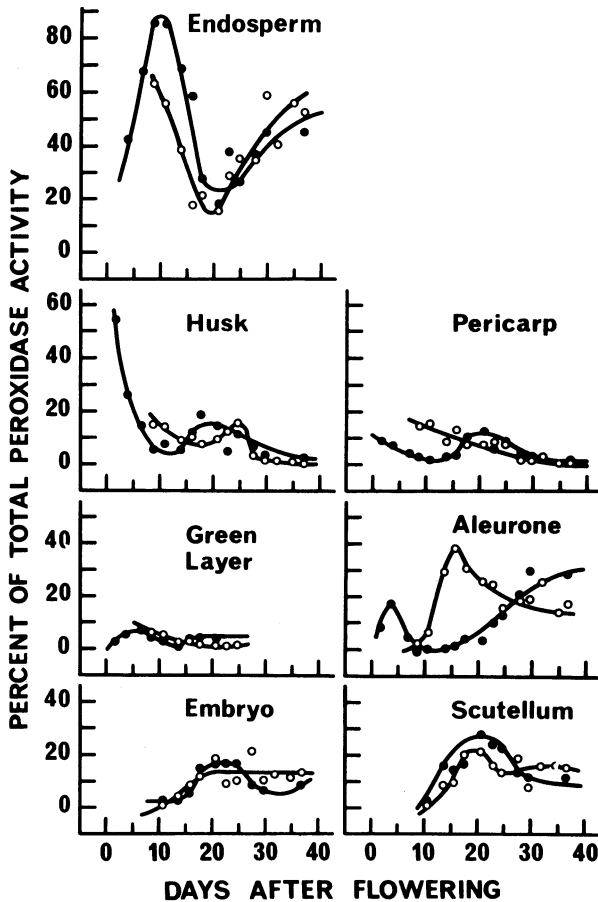


Fig. 3. Changes in the distribution of peroxidase activity in different kernel tissue fractions at various stages of kernel development: o—o Bonanza barley, ●—● Centennial barley.

proportion of peroxidase activity in the aleurone tissue 15 days after anthesis increased to 30% of the total activity in fully mature kernels.

Studies were performed to compare the heat stability of peroxidase activity in six-rowed and two-rowed malting barley. A previous study (7) demonstrated the heat stability of peroxidase isozymes of wheat. The heat stability of barley peroxidase enzymes is shown in Fig. 4. Approximately 50% of the peroxidase activity remained after heating at 70°C. The activity of Bonanza barley was more heat labile than that of Betzes barley. Barley and wheat peroxidase activities have similar resistance to heat denaturation (7).

#### DISCUSSION

At different stages of kernel development, changes in peroxidase activity of the various barley tissues differ markedly from those observed during maturation of wheat kernels (6). Wheat peroxidase activity is localized primarily in the pericarp and green layer tissues during the early stages of kernel development, while that associated with barley pericarp and green layer tissues is extremely low at all stages of kernel development. In addition, peroxidase activity of wheat endosperm tissue is low initially and increases slightly as the kernels ripen, whereas this activity is generally higher and more variable at the different stages of kernel development in barley endosperm tissue.

These changes in peroxidase activity in developing barley and wheat kernels undoubtedly reflect the changing biochemical functions of the tissues at different stages of development. The metabolic role of peroxidases in developing grain is obscure. Suggested roles include destruction of hydrogen peroxide produced by oxidase or flavoprotein-catalyzed reactions and removal of toxic amines and phenols (8). The large number of isozymes found in barley (2) and wheat (7)

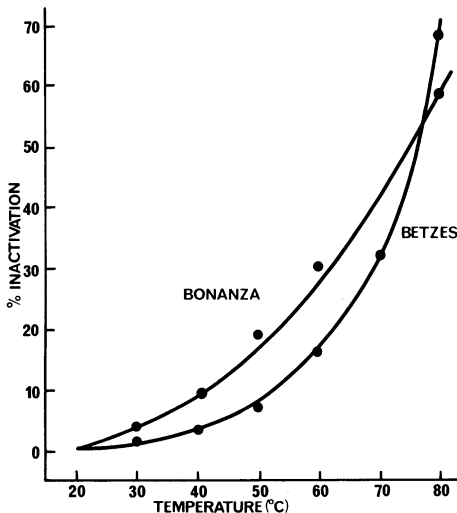


Fig. 4. Effect of 15-min heat-treatment on the peroxidase activity of Bonanza and Betzes barleys.

kernels may suggest different metabolic functions are associated with the different tissues.

The high heat stability of barley peroxidase partially explains the levels of activity present in finished malt and the levels of activity found following high-temperature mashing during the brewing process. Only 25–30% of the peroxidase activity of green malt is lost during kilning (9), while as much as one-third of kilned malt peroxidase may survive mashing temperatures of 70°C for 1 hr (10). The peroxidase isozymes of barley and malt are quite similar (2). Peroxidase activity increases two- to threefold during malting (9), and much of this increase appears to be associated with an increase in endosperm peroxidase activity (11). Very similar characteristics have been found for the properties of peroxidase activity from wheat (7).

Such data may suggest a possible relation between peroxidase activity of kilned malt and beer and quality characteristics of the finished products. Steiner (12) has reported a positive correlation between peroxidase activity of kilned malt and the ratio of soluble to total nitrogen. Steiner also indicated a direct relation between peroxidase activity and the final attenuation limit of beer. Further studies are required to determine the role exerted by peroxidase isozymes of barley, malt, and adjuncts, such as wheat, corn, and rice on the quality of the finished products, and the commercial profitability of altering this enzyme system by plant breeding programs.

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