Changes in Peroxidase Activity and Peroxidase Isozymes of Wheat during Germination[1]

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ABSTRACT

Thirty-two varieties of sound Canadian hard red spring (HRS) and durum wheats were analyzed for peroxidase isozymes by polyacrylamide-slab electrophoresis at acid pH with 3-amino-9-ethyl carbazole as hydrogen donor. Small variations in isozyme patterns were found. Germination of one HRS variety, Manitou, indicated that a fivefold increase in peroxidase activity occurred after 5 days of germination. This was due to increases in amounts of pre-existing isozymes rather than formation of new components. Isoelectric focusing experiments indicated that immature and germinated wheat extracts contained peroxidase isozymes with similar pI values. The anatomical location of some of these isozymes was determined by isoelectric focusing of dissected tissues from immature wheat kernels. Peroxidase activities in extracts of wheat were found to be quite stable to heat with only 50% of the total activity being lost after a 15-min. heat treatment at 70° C.

A previous paper (1) demonstrated that up to 12 peroxidase isozymes were present in extracts of immature wheat kernels. These isozymes were located in different anatomical parts of the kernel and varied in quantitative amounts

[1]Paper No. 338 of the Canadian Grain Commission, Grain Research Laboratory, Winnipeg, Manitoba R3C 3G9, Canada.

throughout kernel development and maturation. Isozymes present in the pericarp and green tissues decreased in intensity as the kernel matured, whereas those present in the aleurone, endosperm, scutellum, and embryo slowly increased. The present study was undertaken to extend the previous work by examining 1) intervarietal differences in peroxidase isozymes from some Canadian HRS and durum wheat varieties; 2) changes in peroxidase activity and peroxidase isozymes of a typical variety during germination; and 3) changes in peroxidase activity and peroxidase isozymes on heating wheat extracts.

Quantitative changes in peroxidase activity were examined using *o*-dianisidine as hydrogen donor for the assay, and changes of isozyme patterns were detected on polyacrylamide slabs using 3-amino-9-ethyl carbazole as hydrogen donor. In addition, extracts of immature and germinated wheat were compared by isoelectric focusing.

MATERIALS AND METHODS

The HRS and durum wheats used for intervarietal comparisons of peroxidase isozymes were from 1971 Plant Breeders' varieties and are listed in Table I. From this material, the varieties Manitou and Hercules were selected for germination

TABLE I. 1971 PLANT BREEDERS' VARIETIES OF WHEAT ANALYZED BY POLYACRYLAMIDE-SLAB ELECTROPHORESIS[1]

Hard Red Spring Wheat		Durum Wheat	
Sample Slot No.	Variety	Sample Slot No.	Variety
1	Marquis	1	Mindum
2	Manitou	2	Stewart 63
3	Neepawa	3	Hercules
4	Thatcher	4	Rolette
5	Cypress	5	D 6062 X D 6142
6	Manitou[2] X RL 4124.1	6	Pelissier
7	Manitou X CT 262	7	Wascana
8	Manitou[6] X RL 4126.2-Chris	8	RL 3601 X (RL 3442-Lakota)
9	Manitou[2] X Giza 144	9	RL 3601 X (RL 3442-Lakota)
10	CT 262 X Manitou	10	RL 3607 X DT 182
11	Manitou X (Reliance-Manitou X 4159.2)	11	RL 3607 X DT 182
12	Manitou[6] X CI 9294	12	RL 3607 X DT 182
13	Canthatch X 4351-331	13	RL 3607 X RL 3656
14	Canthatch X CT 755[2]	14	RL 3607 X RL 3656
15	Atlas 66 X Manitou[2]	15	Blue Giant X Lakota[2]
16	Cypress X CT 244	16	Blue Giant X Lakota[2]
17	Atlas 66-CT 262 X Manitou	17	Blue Giant X Lakota[2]
18	Cypress X CT 244	18	DT 182 X DT 192
19	Park	19	DT 182 X DT 192
20	Parentage unknown	20	DT 182 X DT 192
		21	RL 3601 X (RL 3442-Lakota)
		22	RL 3601 X (RL 3442-Lakota)
		23	DT 182 X DT 192
		24	DT 182 X DT 192

[1]Multiple analyses have been carried out on some varieties of durum wheat which were grown at different locations.

studies. Immature Hercules at 35 days after flowering was obtained from samples collected in 1972 as described previously (1).

Germination of Wheat Kernels

Fifty kernels of wheat were placed in a 13.0 × 13.5-cm. covered sample dish containing two 13.0 × 13.5-cm. sheets of germination paper which had been moistened with 16 ml. water. The samples were then placed in a moisture cabinet in the dark at 23° C. and samples removed after germination times ranging from 1 to 7 days. The germinated samples were stored frozen prior to analysis.

Extraction of Wheat Kernels

Five kernels of germinated wheat were ground in a mortar and pestle with 2 ml. of 12.5% sucrose. The suspension was then centrifuged at 25,000 × g for 10 min., and the supernatant extract was used for the assay. For analysis of Plant Breeders' varieties, 150 kernels were ground in a Moulinex coffee mill, and the ground material was extracted with 10 ml. of 12.5% sucrose in a Virtis "45" homogenizer for 1 min. at medium speed, and then centrifuged as above.

Peroxidase Activity

Enzyme activity was determined as described previously (1) using hydrogen peroxide as substrate and *o*-dianisidine as hydrogen donor.

Polyacrylamide-Slab Electrophoresis and Detection of Isozymes

Electrophoresis was performed at pH 4.75 by the method of MacGregor and Meredith (2) using an Ortec model 4200 slab-electrophoresis system. Isozymes were detected by incubating slabs with 3-amino-9-ethyl carbazole and hydrogen peroxide as described previously (1).

Isoelectric Focusing

Electrofocusing experiments were as described by Vesterberg and Svenson (3) with column, ampholytes and gradient mixer purchased from LKB Products. A 110-ml. column and temperature of 11° C. were used in all experiments. Fifteen kernels of wheat were ground with a mortar and pestle, extracted with 3 ml. water, and the extract mixed into the less dense solution used in forming the sucrose density gradient. Electrofocusing was carried out at 300 v. for 48 to 64 hr. Following electrofocusing, 1.5-ml. fractions were collected from the column in a refrigerated fraction collector, and pH measurements were made at 11° C. on a Sargent model DR pH meter.

RESULTS

Intervarietal Differences in Peroxidase Isozymes

Extracts from 20 varieties of HRS wheat and 12 varieties of durum wheat (Table I, 1971 Plant Breeders' varieties) were subjected to electrophoresis on polyacrylamide slabs and analyzed for peroxidase isozymes with hydrogen peroxide as substrate and 3-amino-9-ethyl carbazole as hydrogen donor. Multiple analyses were also carried out on some durum varieties which were grown at different locations in Western Canada. The results (Fig. 1) indicated that most varieties had identical electrophoretic mobilities with respect to their major peroxidase isozymes but that some variations were evident with the less

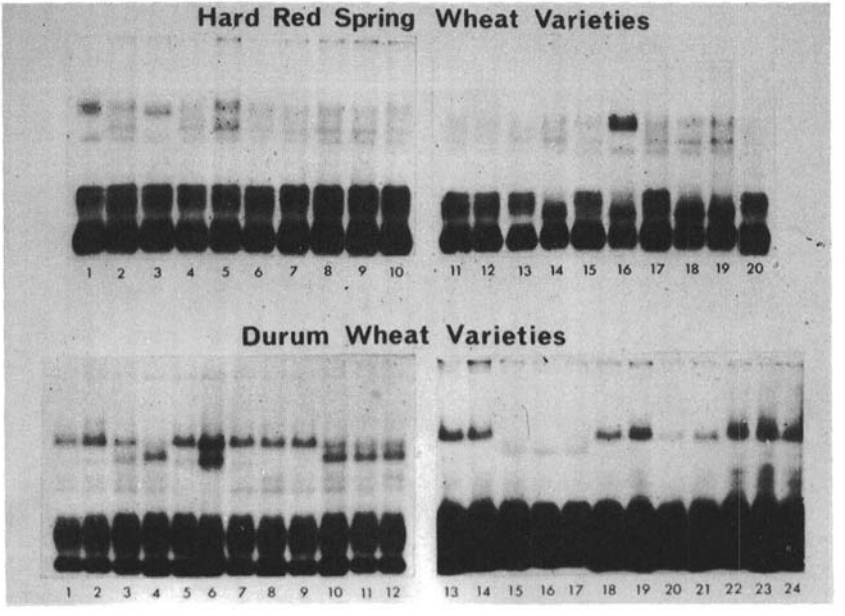

Fig. 1. Peroxidase isozymes in hard red spring and durum wheat varieties from 1971 Plant Breeders' samples. The identity of the samples is given in Table I.

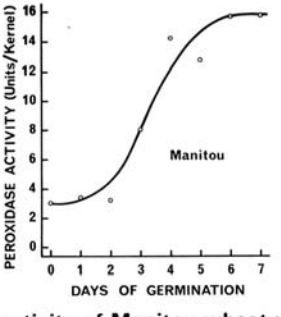

Fig. 2. Change in peroxidase activity of Manitou wheat upon germination.

intensely stained peroxidase isozymes of decreased mobility. This can only be seen in a general way for the varieties in sample slots 13 through 24 because the peroxidase isozymes are so heavily stained. No variations in peroxidase patterns were observed in a durum wheat variety grown at different locations.

In a previous paper (1), it was shown that the intensely stained bands with high mobility were attributable to isozymes present in the endosperm, embryo, and scutellum tissues (in order of decreasing electrophoretic mobility). Two of the HRS wheat varieties, Canthatch X CT 755[2] and Cypress × CT 244, did not contain the major isozyme band found in the scutellum; but Cypress X CT 244 contained instead a very strong band with decreased mobility.

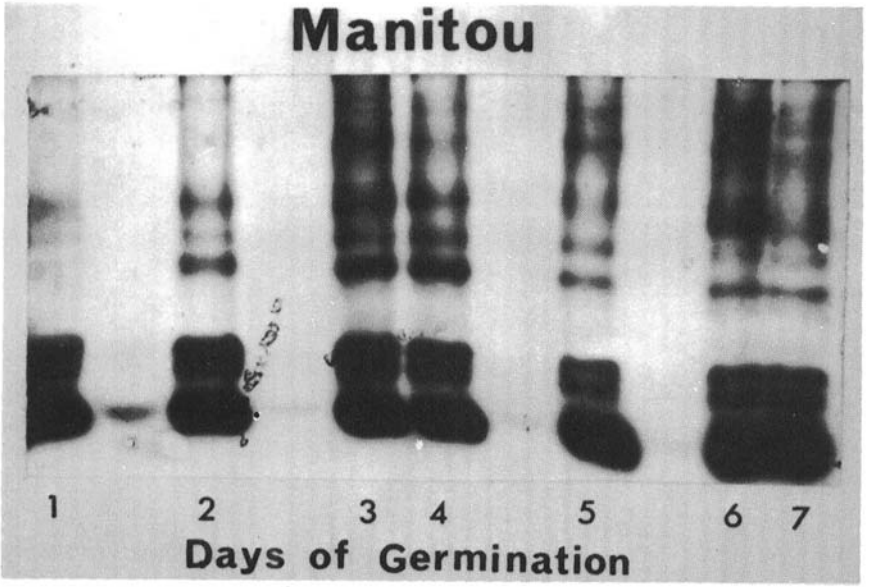

Fig. 3. Changes in peroxidase isozymes of Manitou wheat during germination.

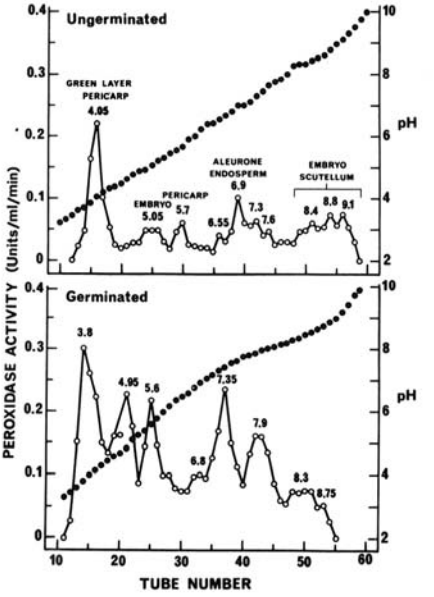

Fig. 4. Isoelectric focusing of peroxidase isozymes from immature and germinated Hercules wheat. The isozymes were identified by electrofocusing extracts of dissected tissues from ungerminated kernels.

Quantitative Changes in Peroxidase Activity and Peroxidase Isozymes during Germination

The variety, Manitou, was selected from among those shown in Fig. 1 as having a typical peroxidase isozyme pattern and was germinated for 7 days. Peroxidase activity with *o*-dianisidine as hydrogen donor (Fig. 2) increased initially after 2 days of germination and developed fivefold after 6 days of germination at which time no further increase was observed.

Examination of the isozyme pattern (Fig. 3) over 7 days of germination indicated that the major peroxidase bands present in the ungerminated and 1-day sample persisted throughout the germination period. A number of minor peroxidase isozymes were found after 3 days of germination which did not change during further germination. These same bands could be discerned, however, in ungerminated samples if the sample size applied was much greater, indicating that it was the intensity of these bands that increased with germination rather than the formation of new peroxidase isozymes. Similar changes in peroxidase isozyme patterns were observed in the durum wheat variety, Hercules, during germination.

Isoelectric Focusing of Wheat Peroxidases

Isoelectric focusing experiments were performed in order to provide further comparisons between the peroxidase isozymes present in sound or immature wheat kernels and those present in germinated wheat. Extracts of immature Hercules wheat at 35 days after flowering and 4-day germinated Hercules wheat were subjected to isoelectric focusing between pH 3 and 10 (Fig. 4). It was found that peroxidase isozymes had widely varying isoelectric points covering almost the entire range from pI 3 to pI 10. Comparison of the isozyme profiles of the immature and germinated wheat demonstrates that the pI values of many of the main peaks were quite similar. Extracts of individual tissues from immature kernels were also subjected to isoelectric focusing in order to identify some of the peaks found in the whole-kernel electrofocusing experiment (Table II). The results provided a tentative identification of some of the individual peaks present in Fig. 4.

TABLE II. ISOELECTRIC POINTS OF PEROXIDASES PRESENT IN ANATOMICAL PARTS OF IMMATURE HERCULES WHEAT KERNELS

Tissue	Electro-focusing Gradient	pI Value												
Embryo	3-10		4.95											
	4-6		5.05											
Scutellum	3-10									8.4	8.9			
	7-10											9.1	9.5	9.7
Endosperm	3-10					6.8								
	3-10		5.15			6.8								
Aleurone	3-10					6.75								
Pericarp	3-10	3.9		5.8										
	3-5	3.95												
Green layer		4.05												
Whole kernels														
Immature	3-10	4.05	5.05	5.7	6.55	6.9	7.3	7.6	8.4	8.8	9.1			
Germinated	3-10	3.8	4.95	5.6		6.8	7.35	7.9	8.3	8.75				

Heat Stability of Peroxidases

Heat stability experiments were carried out on wheat extracts in order to determine 1) whether the peroxidase isozymes of HRS wheat differed from those of durum wheat with respect to heat stability and 2) whether individual peroxidase isozymes of a particular variety might differ in heat lability.

Extracts of mature Manitou and Hercules wheat kernels were placed in water baths for 15 min. at temperatures from 20° to 80° C., rapidly cooled, and assayed for peroxidase activity. From Fig. 5, it is evident that the peroxidase enzymes from both varieties are quite heat stable. For example, approximately 50% of the total peroxidase activity was present after 15 min. treatment at 70° C. Both varieties had very similar heat stabilities. Polyacrylamide slab electrophoresis was used to demonstrate the changes in peroxidase isozyme staining that occurred when extracts were heat-treated at various temperatures. With increasing temperature, the peroxidase bands with decreased mobility slowly disappeared, and at 80° C. only the most intense bands were present. These intense peroxidase bands have been shown to be located in the endosperm, embryo, and scutellum tissues (1).

DISCUSSION

Analysis by polyacrylamide-slab electrophoresis of the peroxidase isozymes present in a number of Canadian HRS and durum wheat varieties indicated that, in most cases, the major isozymes had identical electrophoretic mobilities. In the case of two HRS varieties, Canthatch X CT 755[2] and Cypress X CT 244, however, a major electrophoretic band, present in all the varieties, was missing. In addition, some of the varieties exhibited variance with respect to minor electrophoretic bands.

When Manitou was germinated, the peroxidase activity increased fivefold after 6 days. A threefold increase in activity was reported by Wallerstein et al. (4) after 4.5 days of malting using *o*-phenylenediamine as hydrogen donor to detect

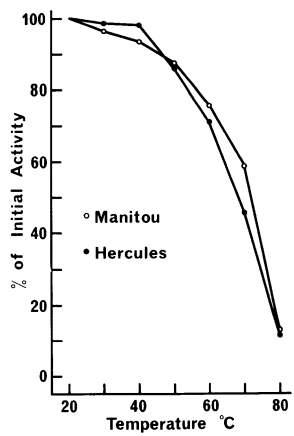

Fig. 5. Effect of 15-min. heat treatment on the peroxidase activity of Manitou and Hercules wheat.

the enzyme. Separation of the peroxidase isozymes by polyacrylamide-slab electrophoresis indicated that pre-existing isozymes increased during germination rather than formation of new isozymes. Comparison by isoelectric focusing of an ungerminated and germinated Hercules wheat also indicated many similarities in the electrofocusing profile of the peroxidases. Daussant and Abbott (5) found, using immunoelectrophoresis, that the antigenic proteins attributable to peroxidase did not appear to be modified up to 13 days from the start of germination. On the other hand, Marchesini et al. (6) and Macko et al. (7) found by polyacrylamide-disc electrophoresis that peroxidase isozymes increased in number from 1 to 5 upon germination. Evans and Mecham (8) have also found by starch-gel electrophoresis that the number of peroxidase isozymes increased from 4 to 7 during germination.

Wheat peroxidase is very heat stable. Thus, heating the enzyme at 70° C. for 15 min. caused only about 50% of the activity to be lost. Even after heating at 80° C. for 15 min. some activity was found; and polyacrylamide-slab electrophoresis indicated that it was the major isozymic components present in the endosperm, embryo, and scutellum that remained. Because this enzyme has high heat stability, peroxidase activity may be important in the breadmaking process.

Acknowledgments

The authors gratefully acknowledge the very capable technical assistance of N. Turriff and F. H. Clements.

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[Received November 28, 1973. Accepted January 28, 1974]