# Specific Aspects of Detection of Peroxidase Isozymes and Their Genetic Control in Barley

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**Abstract**—Identical specimens were separated by electrophoresis in two gels to detect and fix peroxidase isozymes. Both gels were stained by Coomassie brilliant blue for detecting proteins. One gel was previously incubated for detecting peroxidase activity. The differences in electrophoretic patterns between the gels indicate the zones of peroxidase activity. It has been shown that locus *Prx 6H*, controlling a low-mobility grain peroxidase (PRX 6H), is localized to barley chromosome 6. Two loci, *Alb 4H* and *Alb 7H*, controlling the biosyntheses of water-soluble proteins of barley endosperm, were localized to chromosomes 4 and 7. It has been demonstrated that barley species is polymorphic at multiple molecular forms of peroxidase.

It is impossible to obtain a complete isozyme pattern when using the solution containing guaiacol with hydrogen peroxide for incubation of peroxidases. The staining of enzyme-containing bands in gel during the incubation for peroxidase activity disappears in several seconds. The failure in fixation of this enzyme was also reported in the experiments using alkaline buffer system for separation of peroxidase [1].

In this work, we separated peroxidase by polyacrylamide gel electrophoresis of water-soluble proteins in acid medium (pH 3.1), used for separation of wheat gliadin [2]. The difference of our protocol from the method in question was in the absence of urea in the gel. The incubation solution for detecting peroxidase activity contained guaiacol and hydrogen peroxide.

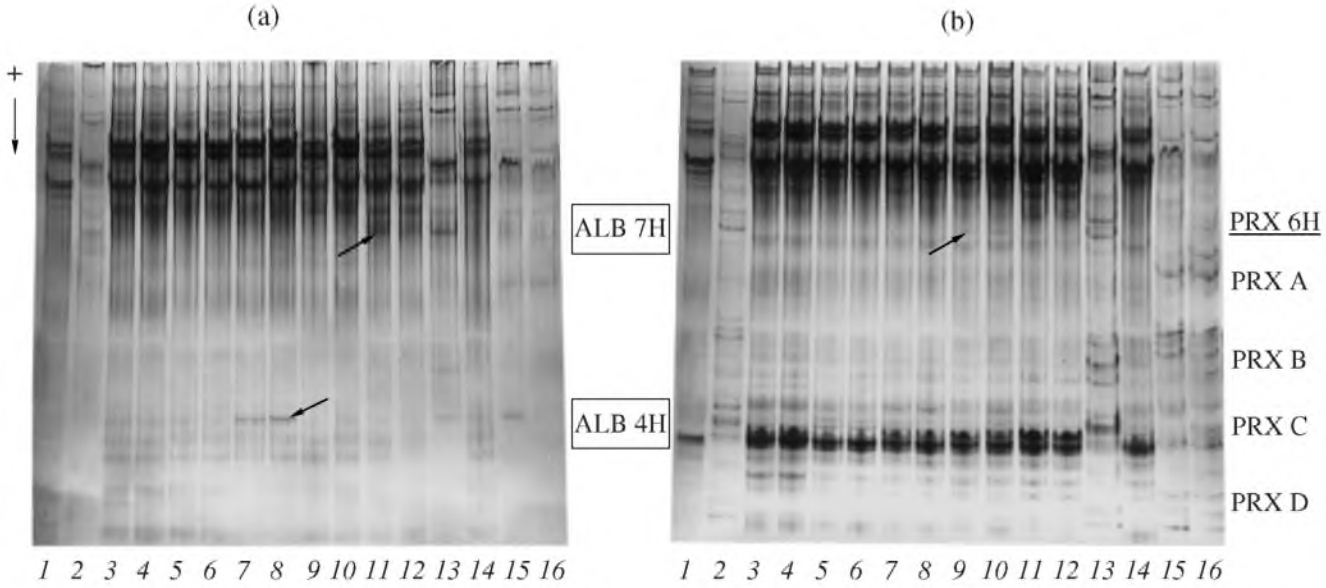
Proteins and enzymes were extracted from mature grains of spring barley cultivars and wheat addition lines carrying individual barley chromosomes.

During this work, we succeeded in finding a simple method for identification of peroxidase activity zones and their fixation on electrophoregrams. Its essence is in comparison of the protein electrophoretic patterns obtained with and without the incubation for peroxidase activity. In both cases, gels are subject to a conventional fixation with TCA and staining with Coomassie brilliant blue R-250. The gel plate after incubation for the enzyme activity also undergoes this procedure. This allows for recording the complete peroxidase pattern (Figure b) on the background of the soluble proteins of mature grain (albumins and globulins; Figure a).

The fractionation of the peroxidase from mature endosperm under acidic conditions (figure, b) yields multiple molecular forms of this enzyme. Barley peroxidase displays a large number of multiple molecular forms and this crop is polymorphic for this enzyme.

Barley varieties (Betzes, California Mariout, and Prato) were shown to differ in several mobility zones detectable when fractionating grain peroxidase under acidic conditions, thereby demonstrating an intraspecies polymorphism of this enzyme.

To determine the chromosomes critical for the genetic control of this trait, we studied wheat–barley addition lines and their parents. The addition lines were bred based on the wheat cultivar Chinese Spring using individual chromosomes of barley cultivar Betzes. The electrophoretic patterns of peroxidases of the wheat lines carrying barley chromosomes 1, 3, 4, and 7 (figure) contained no enzymes of barley cultivar Betzes. However, analysis of the wheat–barley addition lines detected the proteins in the low-mobility zone of barley cultivar Betzes peroxidase (=PRX 6H) in the protein pattern of the wheat line carrying barley chromosome 6 (Figure b, arrow). Thus, we have detected a new locus, designated *Prx 6H* that controls the synthesis of low-mobility barley peroxidase not yet described in literature. The remaining wheat lines carrying barley chromosomes 1, 3, 4, and 7 displayed no barley enzymes in the peroxidase pattern of wheat (Chinese Spring). The absence of the wheat addition lines carrying barley chromosomes 2 and 5 prevented us from answering the question on the chromosome control of other peroxidases from the mature grain detectable under acid conditions designated as PRX A, PRX B, PRX C, and PRX D (figure b). Taking into account these data, we can assume that the products of *Prx4* locus, found in the wheat addition lines carrying barley chromosome 1 [1], are undetectable under the electrophoretic used conditions. It is very likely that barley chromosomes 2 and (or) 5 are involved in the genetic control of PRX A, PRX B, PRX C, and PRX D. At least it is known [3, 4]




Electrophoregrams of water-soluble grain proteins without incubation for peroxidase activity (a) and incubated for peroxidase activity before staining with Coomassie (b): (*1*) wheat cultivar Chinese Spring; (*2*) barley cultivar Betzes; (*3, 4*) wheat addition lines (=AL) with barley chromosome 1H (=AL 1H); (*5, 6*) AL 3H; (*7, 8*) AL 4H; (*9, 10*) AL 6H; (*11, 12*) AL 7H; (*13*) barley cultivar Betzes; (*14*) wheat cultivar Chinese Spring; (*15*) barley cultivar California Mariout 72; and (*16*) barley cultivar Prato (separation in polyacrylamide gel, glycine–acetic acid pH 3.1).

that barley leaf peroxidase is controlled by chromosomes 2 (locus *Prx 2*) and 5 (locus *Prx 1*). No such data have been found for the peroxidase of mature endosperm.

Electrophoretic analysis of water-soluble grain proteins has demonstrated that barley displays polymorphism in these proteins, although not as pronounced as in the case of peroxidase (Fig. 1a). Study of the wheat addition lines carrying individual barley chromosomes allowed us to detect two wheat chromosomes that are critical for the genetic control of two polypeptides (denoted by arrows), designated ALB 7H and ALB 4H. They are located in the low- and high-mobility zones of the electrophoretic pattern (Figure a). The albumin 7H is detectable in the electrophoregrams of grain protein extracts of the wheat addition lines carrying barley chromosome 7. The locus controlling synthesis of this polypeptide was designated *Alb 7H*. The albumin 4H was detected only in the endosperm of the addition lines carrying barley chromosome 4. Correspondingly, the gene providing its synthesis was designated *Alb 4H*.

Thus, we determined the chromosome control of two loci, *Alb 4H* and *Alb 7H*, which code for the biosyntheses of readily soluble proteins of barley endosperm, and localized them to barley chromosomes 4 and 7. We have demonstrated the polymorphism of barley culture in multiple molecular forms of the peroxidase isolated from mature grain and identified by a new approach to detection of enzyme activity. Locus *Prx 6H*, controlling low-mobility form of peroxidase (PRX 6H), was localized to barley chromosome 6.

## REFERENCES

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