Theor. Appl. Genet. 55, 73-76 (1979)

The Chromosomal Location of Peroxidase Isozymes of the Wheat Kernel

C. Benito and M. Pérez de la Vega
Departamento de Genética, Facultad de Biología, Universidad Complutense, Madrid (Spain)

Summary. The analysis of the individual parts of the *Triticum aestivum* L. kernel yields a total of 11 peroxidase isozymes: m, n, a, c, d_1, d, d_2, e, f, g and h (in order from faster to slower migration). Isozymes a, c and d are found in the endosperm (Ed) and seed coats (C), while m, n, d_1, d_2, e, f, g and h are peculiar to the embryo and scutellum (E + S). The use of the nullitetrasomic and ditellosomic series of 'Chinese Spring' wheat allows peroxidase isozymes to be associated with specific chromosome arms. Isozymes a, c and d (Ed) are associated with chromosome arms $7D^S$, $4B^L$ and $7A^S$; whereas isozymes m, d_2, e and f are associated with chromosome arms $3D^S$, $3B^L$, $3D^L$ and $3D^L$, respecitvely. Thus, the E + S isozymes are associated with homoeology group 3 and the Ed isozymes with homoeology groups 7 (a and d isozymes) or 4 (c isozymes).

Key words: *Triticum aestivum* – Wheat, Peroxidases

Introduction

Peroxidases utilize peroxide to oxidize a wide range of hydrogen donors including phenolics, cytocrome-c and nitrite (Felder 1976). Peroxidases are widely distributed among higher plants; they are present in most tissues but they are also frequently organ or tissue specific (Evans and Aldridge 1965; Macdonald and Smith 1972; Scandalios 1964). This is also true for other enzyme systems such as esterases (Macdonald and Brewbaker 1975) and alcohol dehydrogenase (Scandalios 1969).

Studies by Kobrehel and Feillet (1975), and Kobrehel (1978) of the whole wheat kernel revealed five peroxidase isozymes: a, c, d, e and f. These authors assigned the a, c and d isozymes to chromosomes 7D, 4B and 7A, respectively, using the nulli-tetrasomic series of 'Chinese Spring' wheat. Kobrehel (1978), using the ditellosomic series, assigned the same isozymes to chromosome arms $7D^S$, $4B^L$ and $7A^S$, respectively.

The purpose of the present investigation was to locate additional peroxidase isozymes of different parts of the wheat kernel associated with particular chromosome arms.

Materials and Methods

The materials used in this study were the nulli-tetrasomic and ditellosomic series of *Triticum aestivum* L. cv. 'Chinese Spring', supplied by Professor E.R. Sears. The analyses were carried out with individual whole kernels (W.K.), or with parts of individual kernels, specifically the Embryo + Scutellum (E + S), Endosperm (Ed) or Seed coats (C).

Peroxidase isozymes were revealed using horizontal polyacrylamide gel slab electrophoresis. Individual samples were crushed and inmersed in 0.1 M sodium acetate, pH 7.2, for 1 h. 30 min. at 0°C. Small pieces of filter paper were soaked with the liquid and then inserted into 180 × 280 × 2 mm. gels. The gels contained 10% polyacrylamide w/v (95:5 acrylamide:bis-acrylamide in weight) diluted in 0.05 M Tris-ClH buffer, pH 8.6 (Kobrehel and Feillet 1975). The polyacrylamide solution was polymerized by adding 5 ml. of riboflavin solution (3.2 mg. riboflavin, 370 µl. TEMED diluted in 20 ml. of distilled water) and 5 ml. of ammonium persulfate (0.1% w/v) per 140 ml.

The samples were electrophoresed at 25 v/cm. at 4°C for 5 h. 30 min. Isozyme migration was from the anodic to the cathodic side. The gels were stained by the method described by Kobrehel and Feillet (1975).

Results

A total of nine isozymes including m, n, a, c, d, e, f, g and h (in order from faster to slower migration) were identified from whole wheat kernels (W.K.). Isozymes m, n, g and h stained lighter than the other five isozymes (Fig. 1).

When the kernels were excised into three parts (E + S, Ed and C) identical isozyme patterns were obtained for endosperm (Ed) and the seed coats (C) (Fig. 1). Conse-

0040-5752/79/0055/0073/$ 1.00

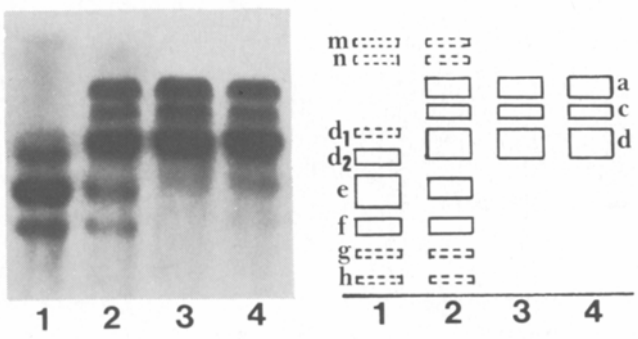

Fig. 1. Peroxidase isozymes of different parts of the wheat kernel: embryo together with scutellum (1), endosperm (3), seed coat (4), and whole kernel (2)

quently we did not separate the endosperm and seed coats in subsequent analyses (hereafter we shall refer to endosperm with its coats by the symbol Ed). However, the embryo pattern (E + S) differed from that of the endosperm. Ed showed three isozymes, a, c and d, whereas E + S had eight isozymes, m, n, d_1, d_2, e, f, g, and h. The m, n, e, f, g and h isozymes had the same migration as those of the whole kernel (W.K.); however, the d_1 (with low stain intensity) and d_2 isozymes migrated faster and slower respectively than the d isozyme (Fig. 1). To avoid mistakes only isozymes with adequate staining intensity were used in location studies.

The results obtained in the nulli-tetrasomic and ditellosomic series are shown in Tables 1 and 2 and in Figures 2 and 3.

The n, d_1, and h isozymes in nulli-2B-tetra-2D and the g isozyme in nulli-3B-tetra-3D stained intensely, the last band was present in nulli-3D-tetra-3B and nulli-3D-tetra-3A (Fig. 2A).

Table 1. Peroxidase isozymes of the wheat endosperm

Stock	a	c	d
Tester	+	+	+
Nulli-7A-tetra-7B	+	+	–
Nulli-7A-tetra-7D	+	+	–
Ditello-7A^S	+	+	+
Ditello-7A^L	+	+	–
Nulli-7D-tetra-7A	–	+	+
Nulli-7D-tetra-7B	–	+	+
Ditello-7D^S	+	+	+
Nulli-4B-tetra-4A	+	–	+
Nulli-4B-tetra-4D	+	–	+
Ditello-4B^L	+	+	+
Critical arm	7D^S	4B^L	7A^S

+ = Isozyme present, – = Isozyme absent

Table 2. Peroxidase isozymes of the wheat embryo and scutellum

Stock	m	d_2	e	f
Tester	+	+	+	+
Nulli-3D-tetra-3A	–	+	+–	–
Nulli-3D-tetra-3B	–	++	+–	–
Ditello-3D^S	+	+	+––	–
Ditello-3D^L	–	+	+	+
Nulli-3B-tetra-3A	+	–	+	+
Nulli-3B-tetra-+D	+	–	++	++
Ditello-3B^S	+	–	+	+
Ditello-3B^L	+	+	+	+
Critical arm	3D^S	3B^L	3D^L	3D^L

+ = Isozyme present, ++ = Isozyme present with a higher intensity, +–– = Isozyme with a very low intensity, – = Isozyme absent

Discussion

In this work we have been able to locate peroxidase isozymes (m, n, d_1, d_2, g and h) additional to the a, c, d, e and f isozymes described by Kobrehel and Feillet (1975) and Kobrehel (1978). This was possible because we

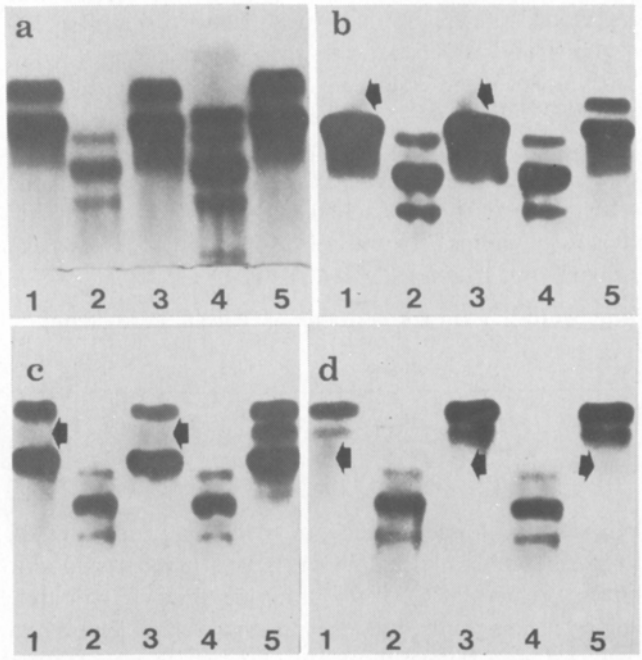

Fig. 2a-d. Wheat kernel peroxidase isozymes of the nulli-tetrasomic and ditellosomic series.
a (1) Nulli-2D-tetra-2B Ed, (2) Nulli-2B-tetra-2A E + S, (3) Nulli-2B-tetra-2A Ed, (4) Nulli-2B-tetra-2D E + S, (5) Nulli-2B-tetra-2D Ed
b (1) Nulli-7D-tetra-7A Ed, (2) Nulli-7D-tetra-7A E + S, (3) Nulli-7D-tetra-7B Ed, (4) Nulli-7D-tetra-7B E + S, (5) Ditello-7D^S Ed
c (1) Nulli-4B-tetra-4A Ed, (2) Nulli-4B-tetra-4A E + S, (3) Nulli-4B-tetra-4D Ed, (4) Nulli-4B-tetra-4D E + S, (5) Ditello-4B^L Ed
d (1) Nulli-7A-tetra-7B Ed, (2) Nulli-7A-tetra-7B E + S, (3) Nulli-7A-tetra-7D Ed, (4) Nulli-7A-tetra-7D E + S, (5) Ditello-7A^L Ed
Arrows indicate isozyme absence

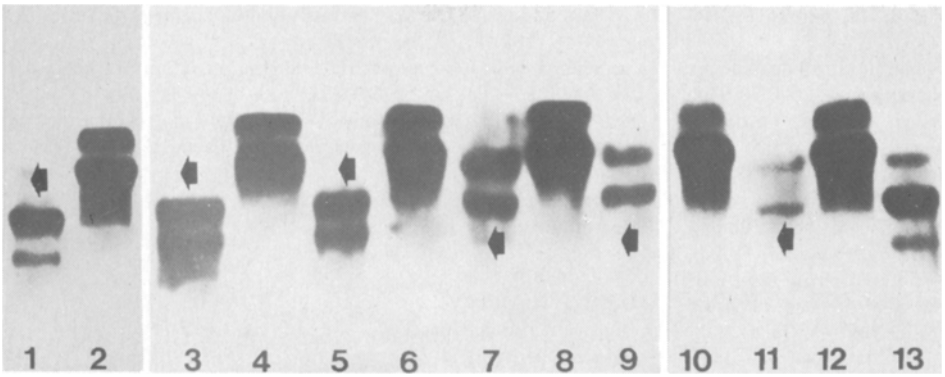

Fig. 3. Peroxidase isozyme of the nulli-tetrasomic and ditellosomic series (1) Ditello-3B^S E + S, (2) Ditello-3B^S Ed, (3) Nulli-3B-tetra-3D E + S, (4) Nulli-3B-tetra-3D Ed, (5) Nulli-3B-tetra-3A E + S, (6) Nulli-3B-tetra-3A Ed, (7) Nulli-3D-tetra-3B E + S, (8) Nulli-3D-tetra-3B Ed, (9) Nulli-3D-tetra-3A E + S, (10) Nulli-3D-tetra-3A Ed, (11) Ditello-3D^S E + S, (12) Ditello-3D^S Ed, (13) Ditello-3D^L E + S

avoided overlapping isozymes (d with d_1 and d_2) when the endosperm (Ed) was analyzed separately from the embryo plus scutellum (E + S), and the concentration of E + S isozymes was greater in the E + S), and the concentration of E + S isozymes was greater in the E + S extract than in the whole kernel extract.

The results given in Tables 1 and 2 establish that peroxidase isozymes m, a, c, d, d_2 and f are associated with different chromosome arms. These results show that isozyme e is associated with the long arm of chromosome 3D. However, it should be noted that this isozyme stained much lighter in nulli-3D-tetra-3A, nulli-3D-tetra-3B and ditello-3D^S, which indicates that one or more genes in one or more additional chromosomes affect isozyme e. The a, d and h isozymes are associated with chromosome 2D, because: (1) they appear with a great stain intensity in nulli-2B-tetra-2D, (2) they have normal intensity in nulli-2B-tetra-2A, (3) they are not observed in nulli-2D-tetra-2A, nulli-2D-tetra-2B and the two ditellosomic of chromosome 2D. However, if the low stain intensity of these latter isozymes is taken into account, it is not possible to be absolutely certain of the relationship between isozyme-absence and gene-absence. Likewise, the g isozyme is present in nulli-3D-tetra-3B, nulli-3D-tetra-3A and ditello-3D^S, but it appears more intensely stained in nulli-3B-tetra-3D.

From the results obtained by us, relating the absence of a peroxidase isozyme with the absence of a chromosome arm, it has been possible to relate several peroxidase isozymes to several chromosome arms. The results obtained for isozymes a, c and d coincide with the data reported by Kobrehel and Feillet (1975) and Kobrehel (1978). On the other hand, Kobrehel and Feillet (1975) indicate the possibility of the e isozyme being related to the A genome; we have not found any relationship between the e isozyme and the A genome (although we did not have available stocks of nulli-2A-tetra-2B, nulli-2A-tetra-2D, nulli-4A-tetra-4B and nulli-4A-tetra-4D). To the contrary, our results show a clear relationship with the D genome. Gale and Spencer (1974) analyzed the total peroxidase activity of endosperm half-grains of ditellosomic series of 'Chinese Spring'. They pointed out that peroxidase activity decreased when either the long arm of chromosome 7D or the short arm of chromosome 7A was absent, although normal activity was maintained when the short arm of chromosome 4B was missing. The last two cases coincide with our results that isozymes d and c (typical of Ed) are associated with chromosome arms 7A^S and 4B^L. On the other hand, the low peroxidase activity related by Gale and Spencer to the absence of chromosome arm 7D^L does not coincide with our results. According to our results the activity should be diminished when the short arm of chromosome 7D is absent but not when the long arm is absent, since the a isozyme (also typical of Ed) is associated with chromosome arm 7D^S.

Separate analyses of the individual parts of the wheat kernel have given us a better understanding of the chromosomal location of these peroxidase isozymes. Also, by using the ditellosomic we have been able to locate most of the genes coding for these isozymes. Verification of the inheritance of these peroxidase isozymes is now under way and will be presented in a future paper.

Acknowledgment

We thank Dr. F. Garcia-Olmedo and Dr. A.L. Kahler for his helpful criticism; and J. Salinas and E. Wiltshire for their technical assistance.

Literature

Evans, J.J.; Aldridge, N.A.: The distribution of peroxidases in extreme dwarf and normal tomato (*Lycopersicon esculentum*). Phytochemistry 4, 499-583 (1965)

Felder, M.R.: Genetic control of four cathodal peroxidase isozymes in barley. J. Hered. 67, 39-42 (1976)

Gale, M.D.; Spencer, D.: Location of genes controlling enzyme production in endosperm. EWAC Newsletter 4, 16-17 (1974)

Kobrehel, K.: Identification of chromosome segment controlling the synthesis of peroxidase in wheat seeds and in transfer lines with *Agropyron elongatum*. Can. J. Bot. 56, 1091-1094 (1978)

Kobrehel, K.; Feillet, P.: Identification of genomes and chromosomes involved in peroxidase synthesis of wheat seeds. Can. J. Bot. 53, 2336-2344 (1975)

Macdonald, T.; Brewbaker, J.L.: Isozyme polymorphism in flowering plants. 5. The isoesterases of maize: tissue and substrate specificities, and response to chemical inhibitors. Tech. Bull. Hawaii Agric. Expl. Sta. 89, 1-24 (1975)

Macdonald, T.; Smith, H.H.: Variation associated with an *Aegilops umbellulata* chromosome segment incorporated in wheat. 2. Peroxidase and leucine aminopeptidase isozymes. Genetics 72, 77-86 (1972)

Scandalios, J.G.: Tissue-specific isozyme variation in maize. J. Hered. 55, 281-285 (1964)

Scandalios, J.G.: Genetic control of multiple molecular forms of enzymes in plants. A review. Biochem. Genet. 3, 37-79 (1969)

Received January 15, 1979
Communicated by R.W. Allard

Dr. C. Benito
Dr. M. Pérez de la Vega
Departamento de Genética
Facultad de Biología
Universidad Complutense
Madrid-3 (Spain)