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The peroxidase isozymes of the wheat kernel: tissue and substrate specificity and their chromosomal location

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Summary. Peroxidase isozymes were studied in the *Triticum aestivum* L. kernel and in nullisomic-tetrasomic and ditelocentric combinations of 'Chinese Spring' wheat. Analyses were carried out on different parts of dry kernels (embryo plus scutellum and endosperm) using polyacrylamide and starch gel electrophoresis, different electrophoretic buffer systems and various staining methods. The peroxidase isozymes showed a low substrate-specificity and a high tissue-specificity. The embryo plus scutellum and the endosperm always presented different peroxidase patterns. Endosperm peroxidases were associated with chromosome arms 7DS, 4BL and 7AS; whereas the embryo plus scutellum isozymes were related to chromosome arms 3AL, 3BL and 3DS. The different results obtained using various electrophoretic techniques are due to the buffer system used. All staining procedures employed revealed the same peroxidase isozymes.

Key words: *Triticum aestivum* – Peroxidases – Tissue and substrate-specificity – Chromosomal location

Introduction

Peroxidases are widely distributed among higher plants and while they are present in most tissues they are also frequently organ or tissue specific (Evans and Alldridge 1965; McDonald and Smith 1972; Scandalios 1964). They are able to utilize peroxide to oxidize a wide range of hydrogen donors including phenolics, cytocrome-c and nitrite (Felder 1976).

In hexaploid and tetraploid wheats and rye it has been demonstrated that different parts of the dry kernel, such as, embryo plus scutellum and endosperm, and also leaf and root, show different peroxidase patterns (Benito and Perez de la Vega 1979; Asins et al. 1982; Garcia et al. 1982; Salinas and Benito 1984). Chromosomal location studies on wheat, rye and barley clearly indicate that the structural genes for peroxidases from different parts of the dry kernel are located on different chromosomes; these same results have been observed for leaf peroxidases (Benito et al. 1980; Salinas et al. 1985; Salinas and Benito 1984; Bosch et al. 1985; Ainsworth et al. 1984). It has not always been demonstrated, however, that the structural genes for peroxidases are triplicated in hexaploid wheats (embryo plus scutellum and leaf peroxidases). In addition, information on different homoeologous groups in hexaploid wheat and rye has been found (Ainsworth et al. 1984; Bosch et al. 1985).

Peroxidases have been electrophoresed and stained in various works using different electrophoretic buffer systems and substrates. Therefore, the peroxidase patterns observed in the same material by different authors are different and the chromosomes related with the synthesis of peroxidases are not the same.

The purpose of the present investigation was to study peroxidases from different parts of the dry kernel in hexaploid wheat using polyacrylamide and starch gel electrophoresis, different electrophoretic buffer systems and various staining methods, and also to compare chromosomal location results obtained with these different techniques.

Materials and methods

The following plants materials were used: i) the hexaploid wheat *Triticum aestivum* L. cv. 'Chinese Spring' (CS); 22 nullisomic-tetrasomic combinations of 'Chinese Spring' wheat (Sears 1966), including at least one nullisomic for each chromosome except 2A and 4A; ii) ditelocentric stocks of 'Chinese Spring' wheat involving the groups 1, 2, 3, 4, 5, 6 and 7 except 2AS, 4Aβ, 4BS and 7DL.

The analyses were conducted on different parts of individual kernels, specifically on the embryo plus scutellum (E+S) and endosperm (Ed). Individual samples were crushed

and immersed 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 the gels.

Peroxidase isozymes were electrophoresed using horizontal 10% polyacrylamide or 12% starch gel slab electrophoresis. The following buffers systems were used: i) tris-ClH 0.05 M Tris-ClH, pH 8.6, as the gel and electrode buffer (Kobregel and Feillet 1975); ii) Poulik: tris-citric acid (0.015 M, pH 7.75) as the gel buffer and NaOH-boric acid (0.3 M, pH 8.6) as the electrode buffer (Poulik 1957); iii) glycine: tris-glycine (0.2 M, pH 8.7) as the gel and electrode buffer (Scandalios 1969); iv) histidine: histidine-ClH (0.006 M, pH 7.0) as the gel buffer and tris-citric acid (0.043 M, pH 7.0) as the electrode buffer (Brewer and Sing 1970); v) aluminium lactate: aluminium lactate (0.1 M, pH 3.2, 3 M urea) as the gel and electrode buffer (Kobrehel et al. 1972).

In all cases, the isozyme migration was from the anodic to the cathodic side, and only the cathodal peroxidases were studied.

The gels were stained using four different methods: i) with catechol (Kobrehel and Feillet 1975); ii) with o-dianisidine (McDonald and Smith 1972); iii) with benzedine (Scandalios 1969); iiii) with 3-amino-9-ethyl carbazole (Shaw and Prasad 1969).

Results

The results obtained using the four staining methods are the same. When the embryo plus scutellum (E+S) and the endosperm (Ed) of the dry kernel are analyzed with the same buffer systems but different staining procedures the same peroxidase pattern was alway observed (one for the E+S and another for the Ed) and the same peroxidase isozymes; only staining intensity of the isozymes was different. The same result was observed for each buffer system employed. These results were also obtained using polyacrylamide and starch gel electrophoresis. The best results were obtained using the staining method with cathechol in polyacrylamide gel and with 3-amino-9-ethyl carbazole in starch gel. (Fig. 1).

The peroxidase patterns shown by the E+S and the Ed are different in all buffer systems used (Fig. 2). The peroxidase patterns for the E+S using various buffers systems are different, as are these for the Ed (Figs. 1 and 2). The peroxidase patterns shown by the E+S in the Tris-ClH, glycine and Poulik buffer systems using acrylamide gels are the same. They consisted of three major isozymes labelled 3, 4 and 5 from faster to slower cathodal migrations (Figs. 1 and 2). The Ed patterns were the same in these three buffer systems and exhibited three major peroxidases and two other isozymes with a low staining intensity. These isozymes have been designated from 1 to 5 in order of cathodal migration (Figs. 1 and 2). The best resolution was obtained using glycine as a buffer system and catechol for staining (Fig. 1). The E+S pattern found in aluminium lactate had four peroxidases (from 1 to 4) while the Ed pattern only showed one peroxidase isozyme (Fig. 2). When the histidine buffer was used, the E+S pattern contained ten isozymes and the Ed had five bands (Fig. 2).

When the analyses were carried out using starch gels, good results were observed only with the histidine buffer: The E+S pattern consisted of eight peroxidase isozymes while the Ed showed four isoperoxidases (Figs. 1 and 2). The remaining buffer systems employed gave a poor resolution.

The results obtained with the nullisomic-tetrasomic and ditelocentric stocks using different electrophoretic buffer systems and various staining methods are shown in Tables 1 and 2 and in Figs. 3 and 4.

In Tables 1 and 2 the relative staining intensities of the peroxidase isozymes are not indicated, only the presence or lack of a specific peroxidase isozyme.

The chromosomal location results obtained using histidine and polyacrylamide or starch seem to indicate that in starch the relative migration of several peroxidases isozymes (of the E+S or of the Ed) is different. (Tables 1 and 2).

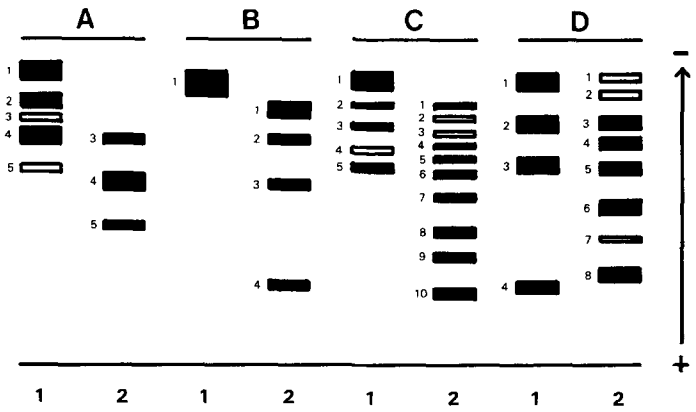

Fig. 1. Diagramatic representation of the peroxidase patterns observed using different buffer systems. *A:* tris-ClH, tris-glycine and Poulik; *B:* aluminium lactate; *C:* histidine; *D:* histidine. *A*, *B* and *C:* 10% polyacrylamide; *D:* 12% starch. 1: endosperm; 2: embryo plus scutellum

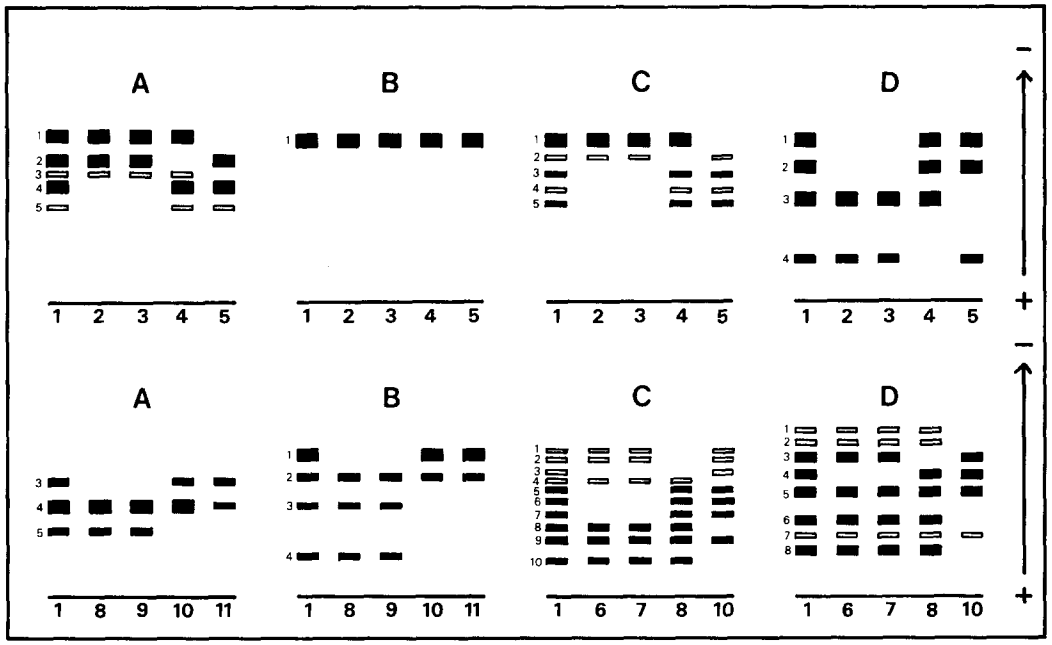

Fig. 2. Diagramatic representation of the peroxidase patterns observed using different buffer systems. *A:* tris-ClH, tris-glycine and Poulik; *B:* aluminium lactate; *C:* histidine; *D:* histidine. *A, B* and *C:* 10% polyacrylamide; *D:* 12% starch. 1: euploid 'Chinese Spring' and ditelocentric lines, 3AL, 3BL, 3DL, 4BL, 7AS, 7DS; 2: N 7A T 7B, N 7A T 7D; 3: 7AL; 4: N 4B T 4A, N 4B T 4D; 5: N 7D T 7A, N 7D T 7B; 6: N 3A T 3B, N 3A T 3D; 7: 3AS; 8: N 3B T 3A, N 3B T 3D; 9: 3BS; 10: N 3D T 3A, N 3D T 3B; 11: 3DS. Top: endosperm; bottom embryo plus scutellum

Table 1. Chromosomal location of wheat endosperm peroxidase isozymes

Stock	Polyacrylamide gels											Starch gels histidine			
	Tris-ClH, Glycine, Poulik					Aluminium lactate	Histidine								
	1	2	3	4	5	1	1	2	3	4	5	1	2	3	4
Tester	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N7A T7B	+	+	+	–	–	+	+	+	–	–	–	–	–	+	+
N7A T7D	+	+	+	–	–	+	+	+	–	–	–	–	–	+	+
7AS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7AL	+	+	+	–	–	+	+	+	–	–	–	–	–	+	+
N7D T7A	–	+	–	+	+	+	–	+	+	+	+	+	+	–	+
N7D T7A	–	+	–	+	+	+	–	+	+	+	+	+	+	–	+
7DS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N4B T4A	+	–	+	+	+	+	+	–	+	+	+	+	+	+	–
N4B T4D	+	–	+	+	+	+	+	–	+	+	+	+	+	+	–
4BL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–

+ = isozyme present; – = isozyme absent

Discussion

The results obtained in this work clearly indicate that the peroxidase isozymes showed a high tissues specificity. The endosperm (Ed) and the embryo plus scutellum (E+S) always displayed different peroxidase patterns.

Similar results have been found previously in several higher plants (Scandalios 1964; Benito et al. 1980; Salinas and Benito 1984; McDonald and Smith 1972; Button et al. 1976; Esen and Soost 1976; Hamill and Brewbaker 1969; Kruger and LaBerge 1974).

All the staining methods used have revealed the same peroxidase pattern. Therefore, it is possible to conclude that peroxidases shown a low substrate specificity and that they can utilize peroxide to oxidize a wide range of hydrogen donors. The same results were obtained when the leaf peroxidases of wheat were studied (Bosch et al. 1985). The substrate specificities of the peroxidase isozymes have been investigated in several higher plants (Liu 1975) and in wheat (Kobrehel et al. 1972; Honold and Stahmann 1968; Laurema 1974).

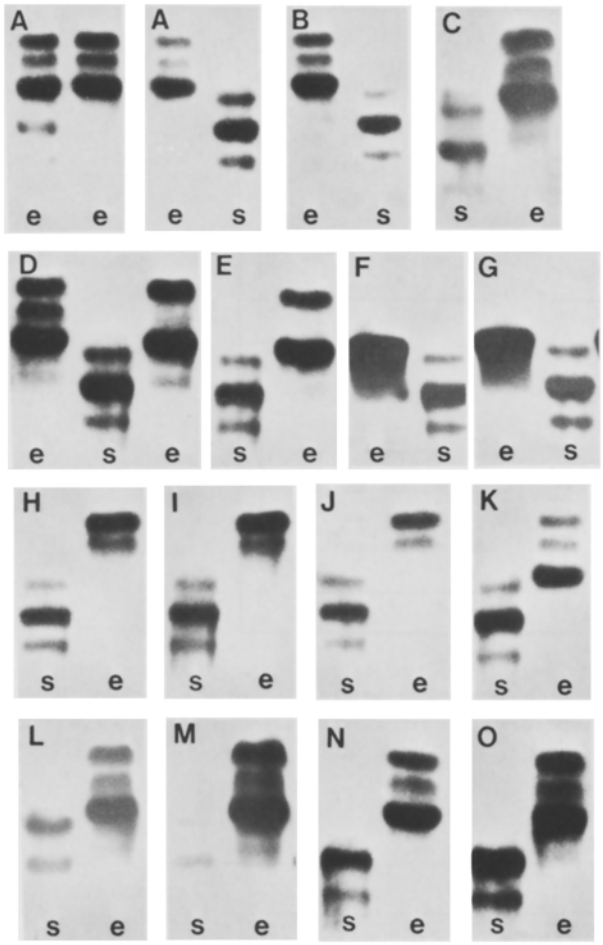

Fig. 3 A–O. Polyacrylamide gels. e: endosperm; s: embryo plus scutellum. **A** Tris-glycine buffer, catechol and o-dianisidine; **B** Tris-ClH buffer, catechol; **C** Poulik buffer, benzidine; **D** Tris-glycine buffer, catechol. From left to right endosperm of euploid 'Chinese Spring'; embryo plus scutellum and endosperm of N4B T4D; **E** Tris-ClH buffer, 3-amino-9-etyl-carbazole, N4B T4A; **F** Poulik buffer, catechol, N7D T7A; **G** Poulik buffer, o-dianisidine, N7D T7B; **H** Tris-glycine, catechol, N7A T7D; **I** Tris-ClH, benzydine, N7A T7D; **J** Poulik, o-dianisidine, N7A T7D; **K** Tris-glycine, o-dianisidine, euploid 'Chinese Spring'; **L** Poulik, o-dianisidine, N3D T3B; **M** Tris-ClH, catechol, 3DS; **N** Tris-glycine, benzidine, N3B T3A; **O** Tris-ClH, catechol, N3B T3D

The chromosomal location of the peroxidase isozymes of wheat suggests that the different staining methods resolved the same peroxidase isozymes. In all cases, the Ed peroxidases have been associated with chromosome arms 7AS, 4BL and 7DS and the E+S peroxidases have been related to chromosome arms 3AL, 3BL and 3DL. The different staining procedures only produced minor changes in the relative staining intensities of the peroxidases. However, the different electrophoretic buffers systems used gave varying results and the differences in resolution power was very distinct.

The best results have been obtained with polyacrylamide gel, tris-glycine and catechol for the Ed peroxidases and with starch gel, histidine and 3-amino-9-ethyl carbazole for the E+S peroxidases. The electrophoretic buffer system is very important because with the appropriate system it is possible to resolve and to locate new peroxidase isozymes.

In our case, using the tris-ClH, tris-glycine and Poulik systems it is possible to resolve three bands in the E+S, but it has only been possible to locate the structural genes for E+S peroxidases 3 and 5 on chromosome arms 3BL and 3DL, respectively. Similar results have been obtained by Benito and Perez de la Vega (1979). However, when we have used histidine as a buffer system we observed ten (polyacrylamide) or eight (starch) peroxidase isozymes and these isozymes were related to chromosome arms 3AL, 3BL and 3DL.

Therefore, the different peroxidase patterns found by different authors and also the different results observed in the chromosomal location of peroxidase structural genes are mainly due to the electrophoretic buffer system employed. Leaf peroxidases have been associated with the 1BS and 1DS chromosome arms by Ainsworth et al. (1984), but Bosch et al. (1985) have associated them with the 2AS, 2BS and 2DS chromosome arms. Moreover, McDonald and Smith (1972) located the structural gene for a leaf peroxidase on the 6BS chromosome arm. These authors employed different buffer systems. In addition, in these three works it has not been possible to locate all the peroxidase isozymes observed.

The genes controlling isozyme phenotypes in hexaploid wheat are, in many cases, triplicate genes that occur as homo-alleles in the three genomes (Hart 1979, 1983). Our results support the hypothesis that the E+S peroxidases are controlled by a triplicate set of homoeologous genes located on the long arms of the group 3 chromosomes (Per-3 set). The leaf peroxidases studied by Ainsworth et al. (1984) are most likely also coded by a triplicate set of structural genes located on the short arms of the group 1 chromosomes (Per-1 set). The leaf peroxidases studied by Bosch et al. (1985) are coded by a triplicate set located on the short arms of the group 2 chromosomes (Per-2 set).

In this work, the Ed peroxidases have been associated with chromosome arms 4BL, 7AS and 7DS in all cases except when we have used aluminium lactate as the buffer system. In this system the Ed had only one band, and probably this band represents different peroxidase isozymes with the same migration.

The Ed peroxidases have been previously associated with 7AS, 4BL and 7DS chromosome arms by Kobrehel and Feillet (1975); Kobrehel (1978) and Benito and Perez de la Vega (1979). These authors have proposed the existence of a translocation between the 7BS and 4BL chromosome arms. Therefore, the Ed peroxidases are also controlled by a triplicate set located on the 7AS, 4BL and 7DS chromosome arms (Per-4 set).

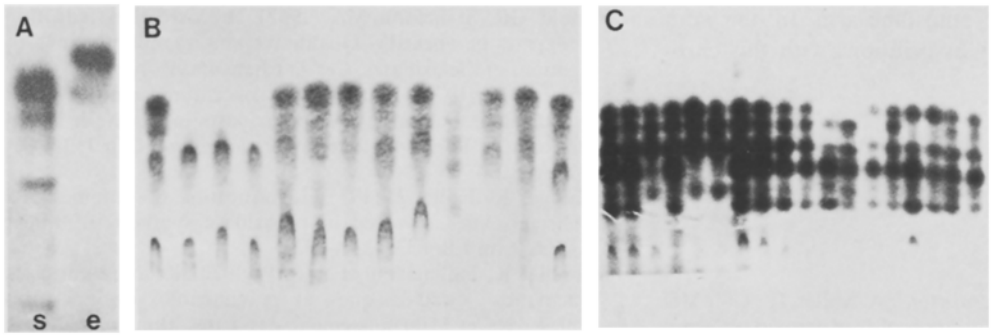

Fig. 4. **A** Polyacrylamide gel, aluminium lactate buffer. s: embryo plus scutellum of euploid 'Chinese Spring'; e: endosperm of euploid 'Chinese Spring'. **B** Starch gel. Histidine buffer. Endosperm of different nulli-tetrasomic and ditelocentric lines of 'Chinese Spring'. From left to right: 1 = euploid 'Chinese Spring' 2 = N7A T7B, 3 = N7A T7D, 4 = 7AL, 5 = 7AS, 6 = N7D T7A, 7 = N7D T7B, 8 = 7DS, 9 = euploid 'Chinese Spring', 10 = embryo plus scutellum of euploid 'Chinese Spring', 11 = N4B T4A, 12 = N4B T4D, 13 = 4BL. **C** Starch gel. Histidine buffer. Embryo plus scutellum of different nulli-tetrasomic and ditelocentric lines of 'Chinese Spring'. From left to right: 1 and 2 = euploid 'Chinese Spring', 3 = 3DS, 4 = 3DL, 5 = N3D T3B, 6 = N3D T3A, 7, 8 and 9 = euploid 'Chinese Spring', 10 = 3BL, 11 = N3B T3D, 12 = N3B T3A, 13 = 3AS, 14 = 3AL, 15 = N3A T3D, 16 = N3A T3B, 17 and 18 = euploid 'Chinese Spring'

Table 2. Chromosomal location of peroxidase isozymes from the wheat embryo plus scutellum

Stock	Polyacrylamide gels																Starch gels histidine							
	Tris-ClH, Glycine, Poulik			Aluminium lactate				Histidine																
	3	4	5	1	2	3	4	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8
Tester	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N3A T3B	+	+	+	+	+	+	+	+	+	–	+	–	–	–	+	+	+	+	+	+	–	+	+	+	+
N3A T3D	+	+	+	+	+	+	+	+	+	–	+	–	–	–	+	+	+	+	+	+	–	+	+	+	+
3AS	+	+	+	+	+	+	+	+	+	–	+	–	–	–	+	+	+	+	+	+	–	+	+	+	+
3AL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N3B T3A	–	+	+	–	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
N3B T3D	–	+	+	–	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
3BS	–	+	+	–	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
3BL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N3D T3A	+	+	–	+	+	–	–	+	+	+	–	+	+	+	–	+	–	–	–	+	+	+	–	+	–
N3D T3B	+	+	–	+	+	–	–	+	+	+	–	+	+	+	–	+	–	–	–	+	+	+	–	+	–
3DS	+	+	–	+	+	–	–	+	+	+	–	+	+	+	–	+	–	–	–	+	+	+	–	+	–
3DL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ = isozyme present; – = isozyme absent

Table 3. Chromosomal location of the structural genes for wheat peroxidases

Tissue or organ	Chromosome arms	Triplicate set	
Leaf	1BS, 1DS	Per-1	May et al. (1973); Ainsworth et al. (1984)
Leaf	2AS, 2BS, 2DS	Per-2	Bosch et al. 1985
Embryo plus scutellum	3AL, 3BL, 3DL	Per-3	Present article
Endosperm	7AS, 4BL, 7DS	Per-4	Present article; Kobrehel (1978)
Root and leaf	6BS		McDonal and Smith (1972)

In Table 3 the chromosomal location of the peroxidases of hexaploid wheat are summarized. The data contained in this table clearly demonstrates that the peroxidases from the different tissues are coded by structural genes located on chromosomes belonging to different homoeologous groups. Also, these data support the high tissue specificity of the peroxidases.

The chromosomal location results obtained by different authors indicate that there are many structural genes coding for peroxidases and that there are at least four sets of triplicate genes located on the chromosomes of the homoeologous groups 1, 2, 3 and 7/4.

McDonald and Smith (1972) have found data suggesting the presence of a leaf and a root wheat

peroxidase on the 6BS chromosome arm. In our work we have not observed any associations with this chromosome arm.

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