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# Leaf peroxidases – A biochemical marker for the group 2 chromosomes in the Triticinae

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**Summary**

Structural genes for the leaf isozymes of peroxidases (E.C. 1.11.1.7) have been located on chromosome arms 2BS, 2DS and probably 2AS of wheat, 2RS of cereal rye and on chromosome 2H of *Hordeum vulgare*. This character provides a useful genetic marker for these chromosome arms, and the results supports the notion of the conservation of gene synteny groups within the Triticinae.

## 1. Introduction

The genes controlling isozymes in hexaploid wheat and its relatives have proved to be valuable markers in genetic and evolutionary studies with these species. In a relatively limited number of studies, over 76 isozyme structural gene loci have been located on specific chromosomes and chromosome arms of wheat by conducting zymogram studies of the existing aneuploid strains (reviewed in Hart, 1983, 1984; Benito, Figueiras & Gonzalez-Jaen, 1984). These genes are located on 41 of the 42 different wheat chromosome arms, and in many cases it has been demonstrated that the genes occur as homoalleles in the three genomes. To date the only wheat chromosome arms lacking such markers are the short arms of the homoeologous group 2.

The isozyme studies carried out in other genomes occurring in the relatives of wheat have provided a total of 40 loci orthologous to wheat genes with known chromosomal location that have been determined in *H. vulgare* cv. 'Betzes', *Elytrigia elongata* and *Secale cereale* cv. 'Imperial', 'King II' and 'Dakoid' (reviewed in Hart & Tuleen, 1983; Salinas & Benito, 1983, 1984*a*, *b*, 1985 *a*, *b*; Benito *et al.* 1985; Salinas *et al.* 1985).

In this paper, we report our results with leaf peroxidases, which provides a new marker gene for the short arms of the group 2 chromosomes of wheat and some other species in the tribe Triticeae.

## 2. Materials and Methods

The following plant materials were used:

Twenty-two nullisomic-tetrasomic combinations of wheat (Sears, 1966), including at least one nullisomic for each wheat chromosome except 2A and 4A, ditelocentric stocks involving 2AS, 2BL, 2DS and the groups 1 and 6 chromosomes (Sears, 1954).

*Triticum aestivum* L. cv. 'Chinese Spring' (CS), *S. cereale* L. cv. 'Imperial' (I); CS-I disomic addition lines supplied by E. R. Sears (Missouri), CS-I ditelocentric addition lines 2RL, 4RL, 4RS, 5RS, 7RL and 7RS obtained from F. J. Zeller (Munchen). *T. aestivum* L. cv. 'Holdfast' (H), *S. cereale* L. cv. 'King II' (KII), H-KII disomic addition lines procured from J. P. Gustafson (Manitoba), H-KII ditelocentric addition lines except 3RL, 3RS and 7RS provided by C. N. Law (Cambridge). *T. aestivum* L. cv. 'Kharkov' (K), *S. cereale* L. cv. 'Dakold' (D) and K-D disomic addition lines supplied by J. P. Gustafson (Manitoba).

*T. aestivum* L. cv. 'Chinese Spring' (CS), *H. vulgare* L. cv. 'Betzes' (B) and the CS-B disomic addition lines except line 5 obtained from A. K. M. R. Islam (Adelaide).

For isozyme analyses, seeds were germinated on moist filter paper at 21 ∓ 1 °C. Crude extracts were stained by maceration of 12-day-old seedling leaves. Small pieces of filter paper were soaked with the liquid and then inserted into the gels.

The gel consisted of a 12% starch slab (14 cm × 17 cm × 1 cm). The gel buffer was 0·005 M-DL-Histidine HCl adjusted to pH 7.0 with 1 N-NaOH, and the electrode buffer was 0·135 Tris (hydroxymethyl) aminomethane and 0·043 M citric acid pH 7.0. Electrophoresis was carried out at a constant voltage of 160 V for 5.30 h at 2–4 °C. The isozyme migration was from the anodic to the cathodic side. The gels were cut horizontally into four slices (2 mm thick) and each slice was stained by a different method. The following staining procedures for peroxidases

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were used: (i) with catechol (Kobrehel & Feillet, 1975), (ii) with 0-dianisidine (MacDonald & Smith, 1972), (iii) with 3-amino-9 ethyl carbazole (Shaw & Koen, 1968).

Gels were fixed in ethanol-water (1:1) after staining.

The chromosome constitution of the addition lines was verified using the C-banding technique of Giraldez & Orellana (1979). All the addition lines examined had the expected 42 chromosomes of wheat and the appropriate two chromosomes or two specific chromosome arms of rye or barley. Only H-KII addition lines revealed a deletion in the short arm of chromosome 2R. Cytological evidence for this deletion has also been reported by Singh & Robbelen (1976).

## 3. Results and Discussion

The three different staining procedures for leaf peroxidases gave the same results, but the staining method described by Shaw & Koen (1968) was the best. These results indicate that leaf peroxidases showed a low substrate-specificity.

The leaf peroxidases (PER) of euploid 'Chinese Spring', 'Holdfast' and 'Kharkov' consist of nine distinct bands (from 1 to 9), the bands 1 and 4 being somewhat thicker and more intense than the other bands (Fig. 1, Lane 1; Fig. 2, Lanes 1, 17).

All the nullisomic-tetrasomic and ditelocentric stocks of CS examined, except those involving the group 2 chromosomes gave an identical phenotype to that of the euploid. The absence of chromosome 2B, or its short arm, caused the loss of band 7 (Fig. 1, Lanes 2, 3, 4; Fig. 2, Lanes 2, 3, 4). Furthermore, four doses of this chromosome resulted in a strengthening of band 7 relative to euploid (Fig. 1, Lane 6; Fig. 2, Lane 6). These results indicate that this band is coded for by a gene on the short arm of 2B chromosome. The absence of chromosome 2D caused the loss of bands 4, 5 and 6 (Fig. 1, Lanes 5, 6; Fig. 2, Lanes 5, 6). Moreover, four doses of this chromosome resulted in a strengthening of bands 4, 5 and 6 relative to euploid (Fig. 1, Lane 3: Fig. 2, Lane 4). These bands were present with a normal intensity in ditelocentric 2DS (Fig. 1, Lane 7; Fig. 2, Lane 7). These results indicate that the bands 4, 5, and 6 are coded for by genes on the short arm of 2D chromosome. Although band 1 did not completely disappear upon the removal of any group 2 chromosome pair from the wheat genome, its relative staining intensity was decreased when the sum of the doses of chromosomes 2A and 2D was reduced below the four doses present in euploid wheat. Thus band 1 was lighter staining than euploid in nulli 2D tetra 2B (Fig. 1, Lane 6; Fig. 2, Lane 6) (nulli 2A tetra 2D and nulli 2A tetra 2B are not available) and its relative staining intensity was increased when chromosomes 2A or 2D were present in four doses and chromosomes 2D and 2A in two doses respectively (Fig. 1, Lane 2, 3; Fig. 2, Lane 2, 4). The ditelocentric 2AS and ditelocentric 2DS are similar to euploid CS phenotype. It is concluded that the chromosome arms 2AS and 2DS each carry a gene coding for an isozyme with the same mobility which together constitute band 1.

The results obtained by Sears & Sears (1979) indicate that the chromosome arms 2BS, 2DL and 2AL are homoeologous, and that the chromosome arms 2BL, 2DS and 2AS are also homoeologous. However, the chromosomal location of genes controlling peroxidase bands on the short arms of the group 2 chromosomes is a biochemical evidence of homoeology between these chromosome arms. The genes have been assigned the gene symbols *Per-A2* (band 1), *Per-B2*

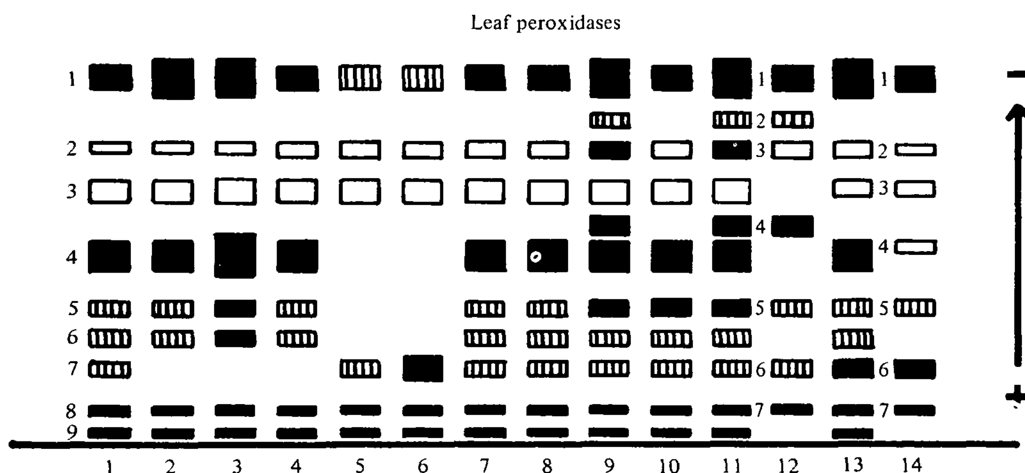


Fig. 1. Leaf peroxidase (PER) zymogram phenotypes of (1) 'Chinese Spring' (CS); 'Holdfast' (H) and 'Kharkov' (K) wheats; (2) nullisomic 2B tetrasomic 2A (N2BT2A); (3) N2BT2D; (4) ditelocentric 2BL (dit 2BL); (5) N2DT2A; (6) N2DT2B; (7) dit 2DS; (8) dit 2AS; (9) 'Chinese Spring–Imperial' (CS–I) wheat-rye disomic addition line 2R (CS–I–2R); (10) 'Holdfast–King II' (H–KII) wheat-rye disomic addition line 2R (H–KII–2R) and ditelocentric addition line 2RS (H–KII–2RS); (11) 'Kharkov–Dakold' (K–D) wheat-rye disomic addition line 2R (K–D–2R); (12) 'Imperial' (I), 'King II' (KII) and 'Dakold' (D) ryes; (13) 'Chinese Spring-Betzes' (CS–B) wheat-barley disomic addition line 2 (CS–B–2); (14) 'Betzes' (B) barley.

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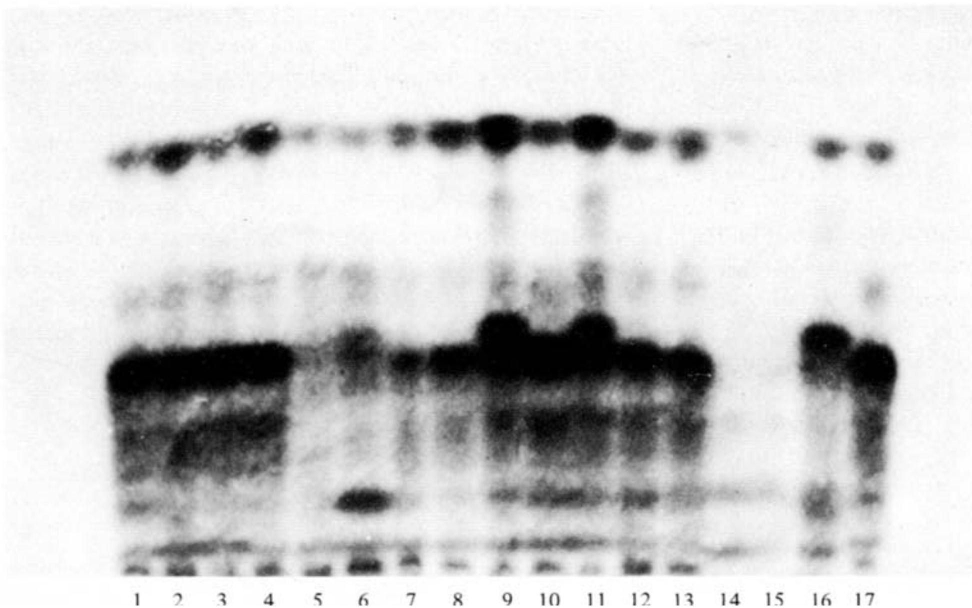


Fig. 2. PER zymogram phenotypes of (1) CS; (2) N2BT2A; (3) dit 2BL; (4) N2BT2D: (5) N2DT2A; (6) N2DT2B; (7) dit 2DS; (8) dit 2AS; (9) CS–I–2R; (10) H–KII–2R or H–KII–2RS; (11) K–D–2R; (12) CS–I–2RL or H–KII–2RL; (13) CS–B–2; (14) B; (15) B; (16) I, KII and D; (17) CS. Samples derived from 12-day-old seedling leaves.

(band 7) and *Per-D2* (bands 1, 4, 5 and 6). The remaining peroxidase bands (2, 3, 8 and 9) could not be related with any particular chromosome.

The structural genes for peroxidases PER-1, PER-2 and PER-3 of the endosperm of mature grains have been located on chromosome arms 7DS, 4BL and 7AS respectively (Kobrehel & Feillet, 1975; Benito & Pérez de la Vega, 1979). Also, the structural genes for embryo and scutellum peroxidases of mature grains have been related with the chromosome arms 3BL and 3DL (Benito & Pérez de la Vega, 1979). MacDonald & Smith (1972) have found that two structural genes for a leaf and a root peroxidase are located on chromosome arm 6BS. Moreover, the structural genes for two leaf peroxidase isozymes have been related with the chromosome arms 1BS and 1DS using acrylamide-gel-slab isoelectric focusing (May, Vickery & Driscoll, 1973). Also, Ainsworth *et al.* (1984) have located the *Per-B1* and *Per-D1* leaf peroxidase loci on the chromosome arms 1BS and 1DS respectively. These authors have found these results using isoelectric focusing. We have not observed any relationship between the leaf peroxidases and the chromosome arms 6BS, 1BS and 1DS. Probably, the reason for these different results is the different buffer systems and electrophoretic procedures used in these studies. On the other hand, the peroxidase bands 2, 3, 8 and 9 of hexaploid wheat have not been related with any particular chromosome, and also Ainsworth *et al.* (1984) have not located the information for five leaf peroxidases. Probably, there are many structural genes for peroxidases in several homoeologous groups and it is possible that many bands encoded by these genes overlap in the gels.

'Imperial', 'King II' and 'Dakold' ryes showed variability for leaf peroxidase phenotype. The rye phenotype has seven bands (from 1 to 7), the bands 1 and 4 being somewhat thicker and more intense than the other bands (Fig. 1, Lane, 12; Fig. 2, Lane 16). The rye peroxidase bands 1, 3, 5, 6 and 7 have the same mobility to the wheat peroxidase bands 1, 2, 5, 7 and 8 respectively (Fig. 1, Lanes 1, 12; Fig. 2, Lanes 1, 16). However, the rye peroxidase bands 2 and 4 have faster mobility than wheat peroxidases 2 and 4 respectively. Six of the seven Chinese Spring–Imperial or Kharkov–Dakold wheat–rye disomic addition lines possessed a leaf peroxidase phenotype similar to that of Chinese Spring or Kharkov. However, addition line 2R (CS–I–2R and K–D–2R) showed all the bands present in Chinese Spring or Kharkov and in addition the rye peroxidase bands 2 and 4 (Fig. 1, Lanes 9, 11; Fig. 2, Lanes 9, 11). Furthermore, the wheat peroxidases 1, 2 and 5 present in this addition line (CS–I–2R and K–D–2R) have stained with greater intensity than those peroxidases of euploid wheat (Fig. 1, Lanes 9, 11; Fig. 2, Lanes 9, 11). Six of the seven Holdfast–King II wheat–rye disomic addition lines possessed a leaf peroxidase phenotype similar to that of Holdfast. However, addition line 2R (H–KII–2R) showed the wheat peroxidase band 5 with a greater staining intensity than that of Holdfast (Fig. 1, Lane 10; Fig. 2, Lane 10). The ditelocentric stocks CS–I–2RL and H–KII–2RL showed the same peroxidase pattern to euploid wheat, whereas the ditelocentric strain H–KII–2RS gave the same peroxidase phenotype as the H–KII disomic addition line 2R.

Therefore, the rye structural genes for peroxidase bands 1, 2, 3, 4 and 5 have been located on chromosome arm 2RS of 'Imperial' and 'Dakold' ryes. The genes have been assigned the gene symbol *Per-R2* (bands 1, 2, 3, 4 and 5). However, only the informa-

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tion for rye peroxidase band 5 has been located on chromosome arm 2RS of 'King II' rye. The existence of a deletion in the short arm of 2R chromosome of the H–KII–2R addition line has been reported by Singh & Robbelen (1976). This deletion could explain the absence of rye peroxidases 1, 2, 3 and 4 in this addition line.

Recently, analysis of wheat-alien addition and substitution lines identified homoeologous loci in rye (*Per-R1*) and barley (*Per-H1*) (Ainsworth *et al.* 1984).

Linkage analyses with rye structural genes for leaf peroxidases have established that the loci controlling the bands 4 and 5 are linked (Figueiras *et al.* 1985). Therefore, our results support this linkage data and suggest that the structural genes for peroxidase bands 1, 2, 3, 4 and 5 could be linked on the chromosome arm 2RS. Probably, the locus *Per-R2* is composed of very tightly linked genes. Linkage analyses with rye structural genes for endosperm peroxidases have established that there exist at least four tightly linked genes located on 7RS chromosome arm (Garcia, Pérez de la Vega & Benito, 1982; Salinas & Benito, 1984*b*).

'Betzes' Barley phenotype consisted of seven bands (from 1 to 7), the bands 1 and 6 being somewhat thicker and more intense than the other bands (Fig. 1, Lane 14; Fig. 2, Lanes 14, 15). The barley peroxidase bands from 1 to 7 had the same mobility to the wheat peroxidase bands 1, 2, 3, 4, 5, 7 and 8 respectively. Five of the six wheat-barley (addition line 5 is not available) addition lines showed the same leaf peroxidase pattern as euploid 'Chinese Spring'. However, addition line 2 showed the wheat peroxidases 1 and 6 with a greater staining intensity than those of euploid CS (Fig. 1, Lane 13; Fig. 2, Lane 13). The barley structural genes for peroxidase bands 1 and 6 have been located on chromosome 2H. The genes have been assigned the gene symbol *Per-H2* (bands 1 and 6). Salinas & Benito (1984*b*) and Salinas *et al.* (1985) using wheat–rye and wheat–barley addition lines have located structural genes for leaf peroxidases on 2RS chromosome arm and on 2H chromosome.

The failure to identify any hybrid bands in the phenotype of wheat–rye addition lines 2R and wheat–barley addition line 2 indicates that leaf peroxidase is probably a monomeric enzyme. Peroxidases are usually characterized by a monogenic control, monomeric behaviour and presence of null alleles (Brown & Allard, 1969 in maize; Marshall & Allard, 1969; Clegg & Allard, 1973 in *Avena*; Felder, 1976 in barley; Benito, Pérez de la Vega & Salinas, 1980 in wheat; Garcia, Pérez de la Vega & Benito, 1982; Pérez de la Vega & Allard, 1984 in rye), although exceptions exist, such as the dimeric rice peroxidases (Endo, 1981).

The location of the genes controlling leaf peroxidases to the short arms of the group 2 chromosomes in wheat, provides the first genetic marker for these chromosome arms. Superoxide dismutase structural genes *Sod-A1* and *Sod-D1* have located on chromosome arms 2Aq (2AL) and 2Dq (2DL) respectively (Neuman & Hart, 1983). The *Per-A2*, *Per-B2* and *Per-D2* genes is a paralogous set of genes located in the short arms of homoeologous group 2 chromosomes. The presence of the structural genes *Per-R2* on 2RS and *Per-H2* on 2H chromosomes gives useful markers for these chromosomes in rye and barley.

The existence of genes controlling a similar biochemical marker on the group 2 chromosomes of wheat, rye and barley is a biochemical evidence of their homoeology, and lends further support to the notion of the conservation of gene synteny groups inherited from the common ancestor of the Triticinae, as proposed by Hart, Islam & Shepherd (1980).

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