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The genetical control of tissue-specific peroxidases, *Per-1*, *Per-2*, *Per-3*, *Per-4*, and *Per-5* in wheat

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Summary. Isoelectric focusing (IEF) of extracts from different tissues of hexaploid wheat cv "Chinese Spring" provided a method of distinguishing and identifying the four known, and one newly discovered, sets of genes encoding peroxidase isozyme production. *Per-1*, carried on the short arms of homoeologous group 1 chromosomes, shows a high degree of conservation and is active in coleoptile tissue. *Per-2*, carried on the short arms of group 2 chromosomes, shows some polymorphism and is most active in root tissue. *Per-3*, on the long arms of group 3 chromosomes, is highly variable and most active in embryo tissue. *Per-4*, carried on chromosome arms *7AS*, *4AL*, and *7DS*, is quite variable and most active in endosperm tissue. (The chromosome nomenclature used in this paper is that agreed to by the 7th International Wheat Genetics Symposium, where the previous designations of *4A* and *4B* were reversed.) Restriction fragment length polymorphism (RFLP)-based maps of the group 7 chromosomes were used to locate *Per-A4* to a distal region of *7AS*. In addition, a further set of genes was identified as being active in root tissue. In wheat a single locus, *Per-D5*, was found on chromosome arm *2DS*.

Key words: Peroxidase – Isoelectric focusing – Hexaploid wheat

Introduction

Peroxidase (E.C. 1.11.1.7) isozymes in hexaploid wheat, *Triticum aestivum*, 2n = 6x = 42, have been extensively studied. The enzyme has low substrate specificity and high tissue specificity (Bosch et al. 1987). Several homoeologous sets of loci controlling peroxidases have been identified independently by various groups employing different plant tissues and a range of electrophoretic techniques. Most of these loci are listed in the wheat gene catalogue (McIntosh 1988) as members of the *Per-1*, *Per-2*, *Per-3*, and *Per-4* gene sets.

The *Per-1* set was identified in extracts from mature leaves and is located on chromosome arms *1BS* and *1DS* (May et al. 1973; Ainsworth et al. 1984). The symbol *Per-2* has been assigned to a set of genes carried on the short arms of homoeologous group 2 chromosomes, identified in analysis of enzymes in young leaves (Bosch et al. 1986). The symbol *Per-3* has been assigned to a set of genes carried on the long arms of homoeologous group 3 chromosomes, identified in analysis of embryo and scutellum tissues (Benito et al. 1980). *Per-4* has been assigned to a set of genes controlling grain peroxidase isozymes identified initially on chromosomes *7A*, *7D*, and *4A* (Kobrehel and Feillet 1975), and subsequently on chromosome arms *7AS*, *7DS*, and *4AL* (Kobrehel 1978). These genes were shown to be endosperm specific by Benito and Perez de la Vega (1979) and Bosch et al. (1987). The set nomenclature was applied to these genes once it became clear that the translocation *4AS.4AL-7BS* could explain the presence of apparent homoeoalleles on chromosomes of different homoeologous groups (Naranjo et al. 1987). One further gene, controlling leaf and root peroxidase isozymes and reported to be on chromosome arm *6BS* by McDonald and Smith (1972), has not yet been assigned a set symbol.

The use of various electrophoretic techniques by the several authors listed above have resulted in *Per-1*, *Per-2*, *Per-3*, and *Per-4* becoming difficult genetic markers for general use in wheat genetic studies. Furthermore, very little evidence of allelic variation between varieties at any

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of these peroxidase loci has been reported, restricting their potential use as markers to wheat-alien chromosome manipulation, rather than in intraspecific genetic studies.

In this paper we report experiments designed to remedy this situation by developing means of identifying the products of the several gene sets with a single electrophoresis system, IEF. Moreover, we have classified a range of wheat genotypes for allelic variation at each of the 11 known loci and at a further locus identified in the course of the work, to identify those with most potential for use in wheat genetic and cytogenetic studies. In one case, *Per-A4*, we have been able to exploit the new RFLP maps of wheat chromosomes to map the locus intrachromosomally, thereby further improving the value of the *Per-4* set as genetic markers.

Materials and methods

Genetic stocks

Aneuploid lines. The available nullisomic-tetrasomic lines (all except N4B-T4A and N4B-T4D) and the relevant ditelosomic (DT) lines of the hexaploid wheat cv "Chinese Spring" ("CS") developed by Sears (1954, 1966a, b) were used to identify the chromosomal control of peroxidase isozymes.

Varieties. Thirty-nine hexaploid genotypes, listed in Table 3, were surveyed for allelic variation. All were obtained from A. J. Worland (Institute of Plant Science Research, Cambridge), including accessions of *T. spelta* (album) (AFRC cereal collection catalogue no. IPSR 1220004), *T. macha* (IPSR 1240005), and Sears' "Synthetic" (IPSR 1190903).

Intervarietal chromosome substitution lines. "CS" ("Timstein") and "CS" ("Hope") developed by E. R. Sears (University of Missouri), "CS" ("Cheyenne") by R. Morris (University of Nebraska), and "Hobbit 'S'" (*T. macha*) and "CS" ("Synthetic") by C. N. Law and A. J. Worland (Institute of Plant Science Research, Cambridge) were used to analyse the chromosomal control of *Per* isozymes not present in "CS".

Recombinant lines and F_2 plants. Forty-three doubled haploid derivatives from the F_1 of the cross "Sicco" × "Highbury" produced by J. W. Snape (Institute of Plant Science Research, Cambridge) were used to determine the position of *Per-A4* on chromosome arm *7AS*. Fifty F_2 plants from the cross of "Hope" × "Timgalen" were used to investigate the nature of "loci" which control the production of several isozymes.

Alien-wheat single chromosome addition lines. The amphiploid and the seven addition lines of "CS"/*Ae. longissima* produced by Feldman (1979a, b) were examined.

Electrophoresis and enzyme visualisation

The embryo ends or the endosperm halves of mature dry grains were used for analysis of *Per-3* and *Per-4*, respectively. Single grain portions were crushed in a microhammer mill and incubated in 40 µl (embryos) or 80 µl (endosperms) of 20% sucrose at room temperature for 1 h and centrifuged briefly prior to application to the gel. Coleoptiles and roots of 5- to 8-day-old etiolated seedlings were used for analysis of *Per-1*, *Per-2*, and *Per-5*, respectively. The coleoptile or roots from a single seedling were ground by pestle in a mortar with 40 µl of 20% sucrose solution at room temperature. The macerate was used immediately for electrophoresis. IEF was performed on 0.25-mm thick, 17-cm wide polyacrylamide gels containing 2% (w/v) ampholyte. The composition of ampholyte and the catholytes and anolytes used were different for different groups of isozymes (Fig. 1); details are given in Table 1. Constant power of 1 W/cm length was applied with a maximum voltage of 3,000 V. Gels were prefocused for 1,000 Vh and samples were loaded upon the gel 1 cm from the anode. Sample wicks were removed at 2,000 Vh and focusing was terminated at 12,000 Vh.

Peroxidase activity was visualised using a modification of the method of Kobrehel and Feillet (1975). The gels were immersed in catechol solution (2.5 g catechol, 1.96 g TRIS, 0.15 g boric acid, 0.19 g ethylenediaminetetraacetic acid (EDTA) and 1.5 g calcium chloride dissolved in 100 ml water) for 10 min. Green-coloured bands appeared immediately after transfer to 0.01 *M* H_2O_2.

Table 1. Ampholytes, catholytes, and anolytes employed for analysis of peroxidase isozymes

Isozyme groups	Ampholyte	Anolyte	Catholyte
I	Isolyte 6–10: Servalyt 9–11 = 2:1	0.33 *M* citric acid	1 *M* NaOH
II, full range	Servalyt 2.5–4: Isolyte 3–10: Servalyt 9–11 = 1:5:1	0.33 *M* citric acid	1 *M* NaOH
III	Servalyte 2–4: Isolyte 3–5 = 1:2	0.04 *M* L-glutamic acid	0.1 *M* NaOH

[a] Isozyme groups are defined in Fig. 1

Results

The peroxidase zymograms of extracts from the four different tissues are shown in Fig. 1. For convenience, these isozymes are defined as groups (I, II, III, and IV), focusing in different pI ranges. The wide pH range gels (Fig. 1A) were employed only for analysis of PER-1 in coleoptiles. The basic pH range gels (Fig. 1B) were used for analysis of PER-2, PER-3, and PER-4. The acidic pH range gels (Fig. 1C) were employed only for analysis of the group labelled PER-5.

The genetical control of several groups of peroxidase isozymes could not be analysed. These included the extremely low pI group seen in root extracts (group IV), most of the low pI activity of coleoptile and root extracts (III), the neutral pI isozymes from roots (II), and the high pI isozymes expressed by both root and coleoptile extracts (I-2).

Per-1

The activity of block II isozymes is weak and can only be observed when gels are heavily loaded (Fig. 1A, coleop-

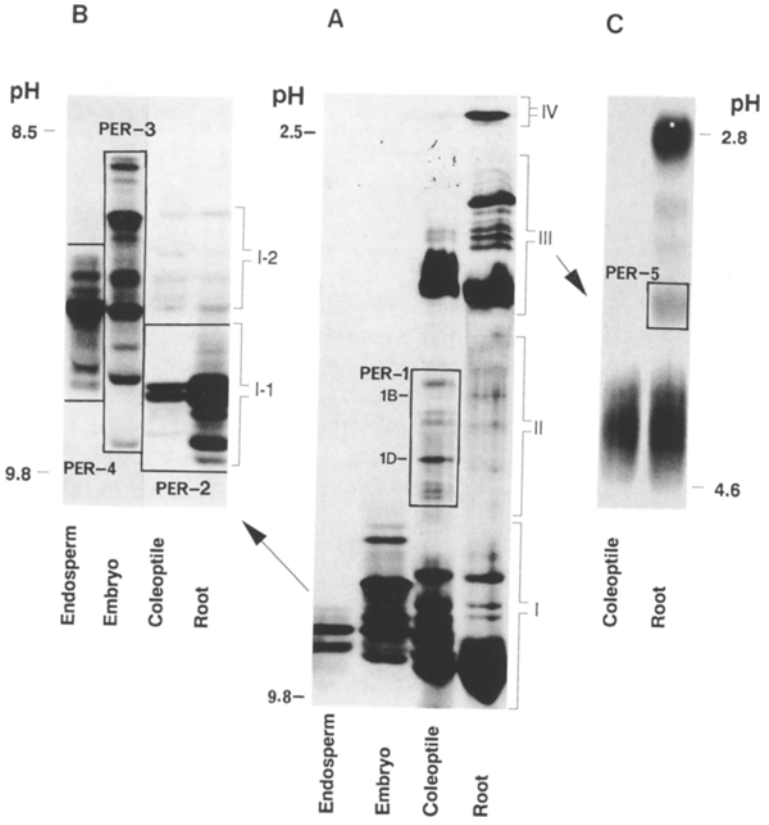

Fig. 1 A–C. The peroxidase isozyme patterns of extracts from different tissues of "CS," **A** showing the relative position of those isozymes extracted from different tissues on wide pH range gels and the two isozymes encoded by *Per-1;* **B** showing the isozymes encoded by *Per-2, Per-3,* and *Per-4* on basic pH range gels; **C** showing those root-specific isozymes and the position of the isozyme encoded by *Per-5*

tile). Nullisomic analysis (not shown) of coleoptile extracts gave similar results to those for leaf extracts reported by Ainsworth et al. (1984). The same genetic effects of both chromosomes *1B* and *1D* were observed, and the *Per-D1b* allelic variant shown by "Synthetic" was confirmed. This group of isozymes appeared to be highly conserved as, of the 39 hexaploid wheat varieties examined, only "Synthetic" showed a different PER-1 isozyme pattern from "CS". In contrast to the conclusion of Ainsworth et al. (1984), our results indicate that these isozymes are not mature leaf specific but are expressed in other green tissues.

Per-2

Root and coleoptile extracts both contained some highly active isozymes focusing around pH 9.5 (group I-1), with a few more lightly stained isozymes of pH around 9.0 (I-2). We were unable to resolve the chromosomal control of the isozymes in I-2 using nullisomic analysis, as none of the nullisomic stocks were deficient for any of these isozymes.

Although both coleoptile and root extracts showed activity around pH 9.0 (I-1), the green tissue showed only a subset of the isozymes observed in roots. The evidence is, however, consistent with both being the products of the *Per-2* set, first described using young leaf tissue by Bosch et al. (1986).

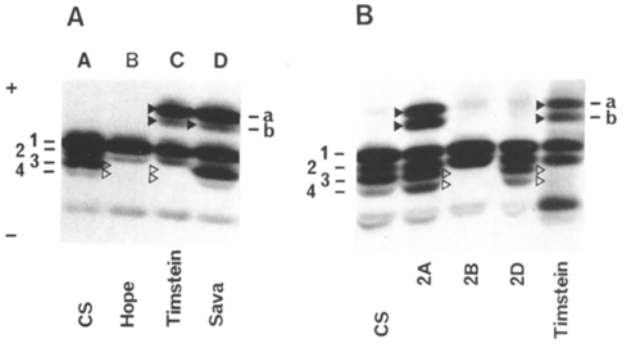

Fig. 2 A and B. Isozyme patterns of PER-2. **A** the four PER-2 phenotypes identified among 39 genotypes, and **B** PER-2 phenotypes of group 2 intervarietal chromosome substitution lines of "CS" ("Timstein"). "*a*" and "*b*" represent isozymes not present in euploid "CS" and marked by ▸. Lack of "CS" isozymes marked by ▹

Nullisomic analysis (not shown) was successful only for root extracts, in which isozymes 3 and 4 in "CS" (Fig. 2 A) were shown to be controlled by a gene(s) on the short arm of chromosome *2B*. These two isozymes were not present in the coleoptile extracts. However some isozymes that were carried by certain genotypes, such as "a" and "b" in "Timstein" (Fig. 2 B), were present in both extracts. Moreover, intervarietal chromosome substitution analysis showed the controlling loci of isozymes

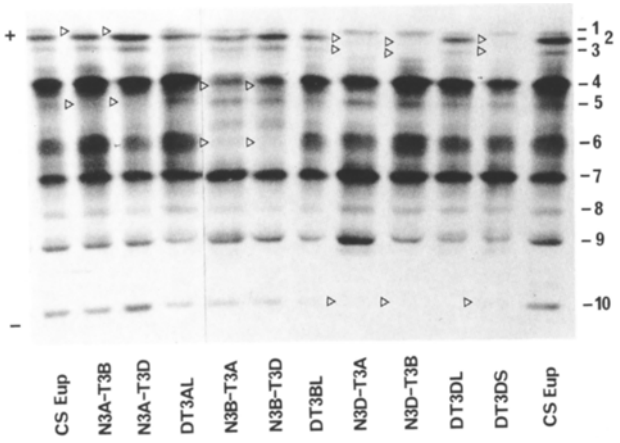

Fig. 3. The PER-3 phenotypes of "CS" group 3 nullisomic-tetrasomic and ditelosomic lines. The absence of "CS" isozymes marked by ▷

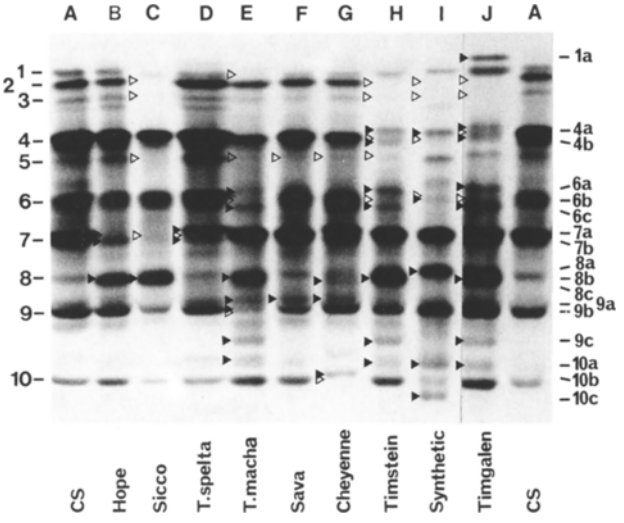

Fig. 4. Ten PER-3 phenotypes identified among a sample of 39 hexaploid genotypes. Isozymes not present on "CS" marked by ▶, lack of "CS" isozymes marked by ▷

"a" and "b" to be on the same chromosome, *2A*, in both tissues. Thus, there was no evidence to suggest that the root peroxidase isozymes were controlled by genes other than *Per-2*.

Allelic variation. Four distinct PER-2 phenotypes were observed in root extracts among the 39 varieties screened (Fig. 2 A). The homoeologous group 2 intervarietal chromosome substitutions of the "CS" ("Timstein") (Fig. 2 B) and "CS" ("Synthetic") series were analysed to identify the *Per-2* loci controlling these differences.

The two isozymes, "a" and "b", were shown to be diagnostic for an allele designated *Per-A2b*, on chromosome *2A* in "Timstein". The absence of isozymes 3 and 4 in "Timstein" and "Synthetic" (not shown) are due to the presence of a null allele, *Per-B2b*, on chromosome *2B*. Confirmation of the *Per-B2b* allele in "Hope" was not possible because the "CS" ("Hope" *2B*) substitution is known to be incorrect (Loegering and Sears 1970).

Per-3

Nullisomic analysis. Embryo extracts of "CS" showed at least ten peroxidase isozymes (Fig. 3). Nullisomic analysis indicated that isozymes 1 and 5 were encoded by chromosome *3A*, isozyme 6 and part of 4 by chromosome *3B*, and isozymes 2 and 3 by chromosome *3D*. Although not consistently observed, isozyme 10 was found to be also encoded by a gene on chromosome *3D*. These genes were further located by ditelosomic analysis to the long arms of these three chromosomes. All three long arm ditelosomic lines (*DT3AL*, *DT3BL*, and *DT3DL*) exhibited an identical zymogram to that of euploid "CS", while the two available short arm ditelosomic lines [*DT3AS* (not shown) and *DT3DS*] exhibited the same zymograms as their respective nullisomic lines.

Allelic variation. The *Per-3* loci proved to be extremely polymorphic. Among the 39 hexaploid wheat lines, 11 PER-3 phenotypes were identified. Ten phenotypes (A-J) are shown in Fig. 4, another (K) represented by "Hobbit 'S'" is shown in Fig. 5 A. Seventeen non-"CS" isozymes were observed. The distribution of these isozymes among the 11 PER-3 patterns is listed in Table 2. Genes encoding 8 of these have been assigned to specific chromosomes by analysis of intervarietal substitution lines: isozymes 4a, 4b, 6b, 8a, and 9c by gene(s) on chromosomes *3B*, 9a and 9b by gene(s) on chromosome *3D*, and 8b by gene(s) on both chromosomes *3B* and *3D* (Fig. 5). Analysis of only the located isozymes allowed 3 alleles to be identified at *Per-A3*, 5 at *Per-B3*, and 7 at *Per-D3*. Three sets of group 3 intervarietal chromosome substitution lines are shown in Fig. 5.

Per-A3 variants. The three alleles identified included: the "CS" prototype *Per-A3a*, which encodes isozymes 1 and 5; *Per-A3b*, an allele in which isozyme 5 was absent, exemplified by "Timstein" (Fig. 5 B); and *Per-A3c*, a null allele lacking both isozymes 1 and 5, exemplified by "Hobbit 'S'" (Fig. 5 A).

Per-B3 variants. The five alleles identified included: *Per-B3a*, the "CS" prototype, characterised by isozyme 6; *Per-B3b*, exemplified by "Hope", which encodes isozymes 6 and 8b; *Per-B3c*, carried only by the *T. macha* accession, which encodes isozymes 8b and 9c; *Per-B3d*, exemplified by "Timstein" (Fig. 5 B), which encodes

Table 2. Isozymes present in each of the 11 PER-3 patterns

Pattern	Isozymes[a]				Representative variety
	Per-3A	Per-3B	PER-3D	Non-located	
A	1,5	4′,6	2,3,10	4′,7,8,9	CS
B	1,5	4′,6,8b	2,3,10,8b	4′,9,7b	Hope
C	1	4′,6,8b	8b′,10	4′,9	Sicco
D	1,5	4′,6′	2,3,10	4′,8,9,7a,7b	*T. spelta*
E	–	8b′,9c	2,3,10,8b′,9a,9b	7,6a,6c,10a	*T. macha*
F	1	4′,6	2,3,10,9a	4′,7,8,9	Sava
G	1	4′,6	2,3,9a	4′,7,8,8c,10b	Cheyenne
H	1	4a,4b,8b′,9c	10,8b′	7,9,6a,6c,10a	Timstein
I	1,5	4a,6b,8a	10	7,9,10a,10c	Synthetic
J	1,5	4a,4b,8b′,9c	10,8b	7,9,1a,6a,6c,10a	Timgalen
K	1	4′,6	2,3,10	4′,7,8,9	Hobbit 'S'

[a] 4′ and 8b′ indicate only part of the activity

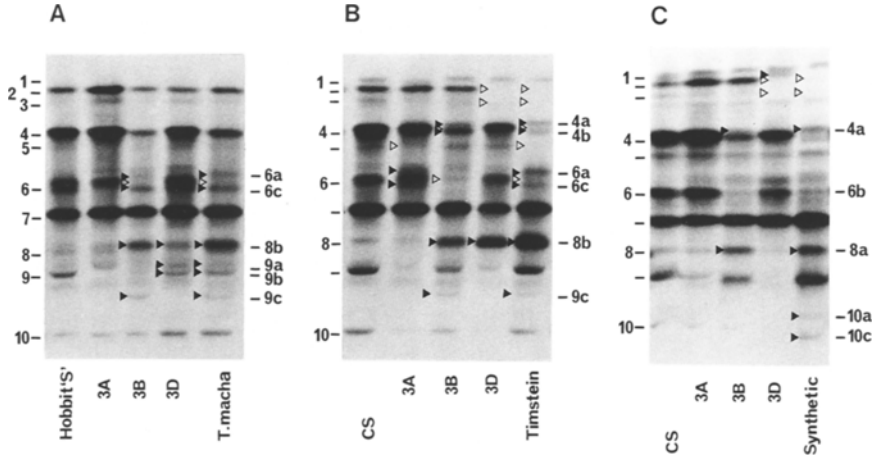

Fig. 5A–C. The PER-3 phenotypes of group 3 intervarietal chromosome substitution lines: **A** "Hobbit 'S'" (*T. macha*); **B** "CS" ("Timstein"), and **C** "CS" ("Synthetic"). ▸ denotes donor parent isozymes and ▷ denotes lack of recipient parent isozymes

isozymes 4a, 4b, 8b, and 9c; and *Per-B3e,* exemplified by "Synthetic" (Fig. 5C), which encodes isozymes 4a, 6b, and 8a. The precise definition of the *Per-B3* allele carried by "Hope" was not possible because the "CS" ("Hope" 3B) substitution is known to be unreliable (E. R. Sears, personal communication). This was demonstrated in the analysis of the malic enzyme locus, *Mal-B3,* by Liu and Gale (1988).

Per-D3 variants. The seven alleles included: the "CS" prototype, *Per-D3a,* encoding isozymes 2, 3, and 10; *Per-D3b,* exemplified by "Hope", which encodes isozymes 2, 3, 10, and 8b; *Per-D3c,* exemplified by "Timstein" (Fig. 5B), which encodes isozymes 10 and 8b; *Per-D3d,* exemplified by *T. macha* (Fig. 5A), which encodes isozymes 2, 3, 10, 8b, 9a, and 9b; *Per-D3e,* exemplified by "Sava", which encodes isozymes 2, 3, 10, and 9a; *Per-D3f,* exemplified by "Cheyenne", which encodes isozymes 2, 3, and 9a; and *Per-D3g,* exemplified by "Synthetic" (Fig. 4), which encodes isozyme 10 only.

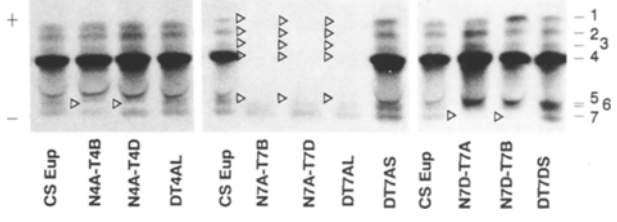

Fig. 6. The PER-4 phenotypes of "CS" groups 4 and 7 nullisomic-tetrasomic and ditelosomic lines. The absence of "CS" isozymes marked by ▷

Per-4

Nullisomic analysis. Endosperm extracts of "CS" showed seven PER-4 isozymes. Nullisomic analysis demonstrated that all of them were encoded by genes on chromosomes *7A, 4A,* and *7D.* Isozymes 1–5 were shown to be encoded by a gene(s) on chromosome *7A,* while each of the other two chromosomes controls only a single

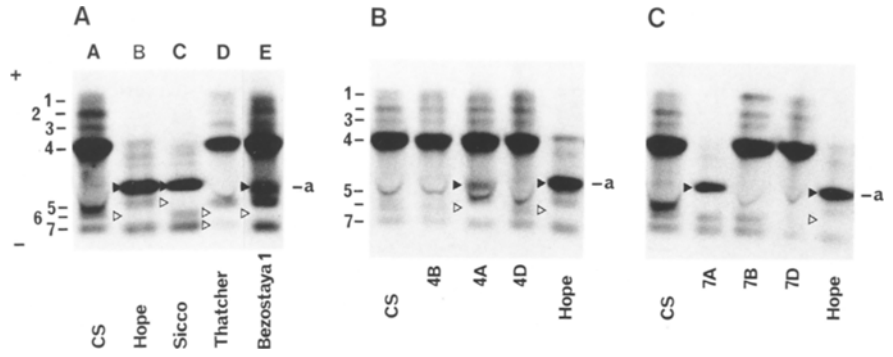

Fig. 7. A The five PER-4 phenotypes identified among a sample of 39 hexaploid wheat genotypes. **B** The PER-4 phenotypes of groups 4 and 7 intervarietal chromosome substitution lines of "CS" ("Hope"). Isozymes not present in "CS" marked by ▸ and lack of "CS' isozymes marked by ▹

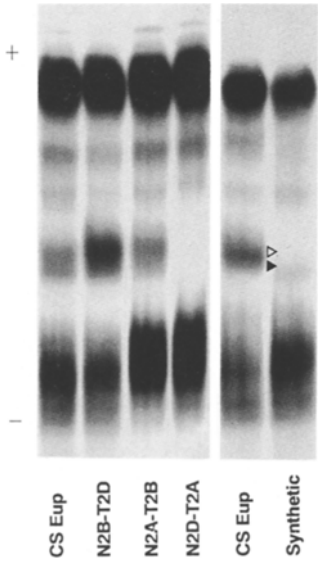

Fig. 8. PER-5 phenotypes of "CS" group 2 nullisomic-tetrasomic and ditelosomic lines and the *Per-D5b* phenotype expressed by "Synthetic". Isozymes not present in "CS" marked by ▸, lack of "CS" isozymes marked by ▹

isozyme. Analysis of ditelosomics confirmed location of the *Per-4* set to chromosome arms *7AS*, *7DS*, and *4AL*, respectively (Fig. 6).

Allelic variation. Five phenotypes (A–E) were observed among the 39 hexaploid wheat lines surveyed (Fig. 7A). The one non-"CS" isozyme, "a", observed among these genotypes was shown to be composed of two isozymes, the major one encoded by a gene on chromosome *7A* and the other by a gene on chromosome *4A*, by the analysis of "CS" ("Hope") group 4 and 7 chromosome substitution lines (Fig. 7B and C).

Based on the classification of genes encoding these endosperm peroxidase isozymes, three alleles were identified at *Per-A4*, three at *Per-B4*, and two at *Per-D4* (Table 3). *Per-A4* variants: *Per-A4a*, carried by "CS", encodes isozymes 1–5. "Hope" carries *Per-A4b*, encoding isozymes 5 and "a", and "Sicco" carries *Per-A4c*, encoding only isozyme "a". *Per-B4* variants: *Per-B4a*, carried by 'CS", encodes isozyme 6; *Per-B4b*, exemplified by "Sicco", encodes part of isozyme a; and *Per-B4c*, carried by "Thatcher", is a null allele. *Per-D4* variants: *Per-D4a*, carried by "CS", encodes isozyme 7, and *Per-D4b*, carried by "Thatcher", is a null allele.

Per-5

Extracts of root tissue produced a few poorly focused peroxidase isozymes in block III. One of them was shown to be encoded by a gene(s) on the short arm of chromosome *2D*, as both *N2D-T2A* and *N2D-T2B* failed to produce this isozyme (Fig. 8), while it was expressed by *DT2DS* (not shown). Although located on the same chromosome arm, this locus is unlikely to be part of *Per-D2*. *Per-2* encodes both root and coleoptile isozymes, while the isozyme(s) encoded by *Per-D5* is root-specific. In addition, the isozymes encoded by *Per-2* have very high pIs, while the single isozyme encoded by *Per-D5* has a very low pI. Nullisomic analysis failed to detect any effect of either chromosome *2A* or *2B* (Fig. 8). As in the case of PER-1, PER-5 was also found to be highly conserved. Of all the 39 varieties screened, only "Synthetic" produced a different isozyme pattern, in which "CS" PER-D5 isozyme was replaced by an isozyme with weaker staining activity and higher pI (Fig. 8).

Although, in wheat, involvement of only a single chromosome was demonstrated for PER-5 production, analysis of wheat-alien chromosome addition lines demonstrated a homoeolocus in *Ae. longissima* (not shown). The identification of two members of the set on chromosomes *2D* and $2S^l$ justifies the designation of *Per-5* to the loci.

Intrachromosomal mapping of Per-A4

The chromosome arm location of *Per-4* loci on *7AS*, *7DS*, and *4AL* indicates that this set of loci must be located distally on these chromosome arms, because cy-

tological (Naranjo et al. 1987) and genetical (Chao et al. 1989) evidence indicate that it is the distal region of the short arm of the "original" *7B* that has been translocated to the long arm of *4A*.

Analysis of the 43 doubled haploid derivatives from the cross "Sicco" (*Per-A4c*) × "Highbury" (*Per-A4a*) allowed segregation at the *Per-A4* locus to be tested against those RFLP loci on the same chromosome arm. Previous results indicate that the linkage value between *XWx* and *XNra* is 11 centimorgans (cM), and that *XWx* is closer to the centromere (Chao et al. 1989). The present analysis (data not shown) provides values: *Per-A4–XWx*, 20.1 ± 6.5 cM and *Per-A4–XNra*, 23.3 ± 6.4 cM, establishing the gene order of these three loci from the centromere as *Per-A4*, *XWx*, and *XNra*. Thus, of all the known markers located in the translocated part of homoeologous group 7 chromosomes, *Per-A4* is nearest to the breakpoint.

The nature of Per-4 locus

Most of the *Per* loci described above have phenotypes that comprise more than one isozyme. The possibility therefore exists that these loci represent genes dispersed over a chromosome arm. In order to investigate this, the segregational analysis of five isozymes segregating at *Per-A4* was carried out for the cross of "Hope" (*Per-A4b*) × "Timgalen" (*Per-A4a*) (not shown). The five isozymes associated with *Per-A4a* always segregated as a block relative to the single isozyme characterising the *Per-A4b*.

Table 3. Allelic variation of peroxidase isozymes at *Per-2*, *Per-3*, and *Per-4* loci

Variety	Genotype								
	A2	B2	D2	A3	B3	D3	A4	B4	D4
CS, Atlas 66, H93-70 Vilmorin 27, Starke, Champlein, Lutescens 62, Cappelle-Desprez, Koga II, Bersee	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
Mara, Carmen, *T. spelta* (album)	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*b*	*a*
Thatcher	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*c*	*b*
Spica	*a*	*a*	*a*	*a*	*b*	*b*	*c*	*b*	*a*
Poros	*a*	*a*	*a*	*b*	*a*	*e*	*a*	*a*	*a*
Highbury	*a*	*a*	*a*	*b*	*a*	*e*	*a*	*b*	*a*
Moulin	*a*	*a*	*a*	*b*	*a*	*f*	*a*	*a*	*a*
Cheyenne, C591	*a*	*a*	*a*	*b*	*a*	*f*	*a*	*b*	*a*
Desprez 80	*a*	*a*	*a*	*b*	*a*	*e*	*a*	*b*	*a*
Sicco	*a*	*a*	*a*	*b*	*b*	*c*	*c*	*b*	*a*
Purple Pericarp	*a*	*b*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
Karcag, RL4137, Favorits, Bezostaya 1	*a*	*b*	*a*	*a*	*a*	*a*	*a*	*b*	*a*
Hope	*a*	*b*	*a*	*a*	*b*	*b*	*b*	*b*	*a*
Synthetic 6 ×	*a*	*b*	*a*	*a*	*e*	*g*	*a*	*a*	*a*
Rendezvous	*a*	*b*	*a*	*b*	*a*	*e*	*a*	*b*	*a*
Huntsman	*a*	*b*	*a*	*b*	*a*	*f*	*a*	*b*	*a*
Hobbit 'S', Glennson, VPM 1	*a*	*b*	*a*	*c*	*a*	*a*	*a*	*b*	*a*
T. macha	*a*	*b*	*a*	*c*	*c*	*d*	*a*	*b*	*a*
P 168	*b*	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
Sava	*b*	*a*	*a*	*b*	*a*	*e*	*a*	*a*	*a*
Timgalen	*b*	*b*	*a*	*a*	*d*	*c*	*a*	*a*	*a*
Timstein	*b*	*b*	*a*	*b*	*d*	*c*	*a*	*b*	*a*

Discussion

In this paper we have confirmed the location of the four homoeologous sets of peroxidase loci in wheat, defined the chromosomal location of one further set, *Per-5*, and identified several groups of isozymes which, at some later date, may yield evidence of even more *Per* loci. Like Bosch et al. (1987), we were unable to confirm the location of any peroxidase loci on chromosome *6BS* as reported by McDonald and Smith (1972).

Previously, most published methods of analysis have considered only single sets of *Per* genes at a time, and have required a range of electrophoretic techniques. This has led to some difficulties, e.g., Bosch et al. (1986), while describing the *Per-2* system in leaf extracts using starch gels, were unable to visualise the *Per-1* system in the same material. The *Per-3* and *Per-4* systems have, however, been visualised in analysis of embryo and endosperm tissues using both polyacrylamide and starch gels by Bosch et al. (1987). Here we have described a single electrophoretic system in which all the products of all of the various known sets of peroxidase genes can be distinguished and analysed.

The present analysis has revealed considerable, previously unreported, variation at several of the *Per* loci. Only limited variation at *Per-D1* (Ainsworth et al. 1984) and at the *Per-3* and *Per-4* loci (Bonito et al. (1980) has been noted previously. It is clear from the range of alleles and allelic frequencies among varieties (Table 3) that some loci are particularly variable and, therefore, potentially very useful for marker-aided applications in breeding and genetic analysis. Variation was detected at ten loci, with only *Per-B1* and *Per-D2* found to be completely conserved in the varieties screened. *Per-B2*, *Per-A3*, *Per-D3*, and *Per-B4* were particularly variable. Inspection of the data for all the hexaploid bread wheat varieties (ignoring "Synthetic" and the *T. spelta* and *T. macha* accessions) showed that around 50% of pairwise comparisons involved allelic differences for each of these four loci. A similar analysis of RFLP loci by Chao et al. (1989) revealed only 4 loci out of 54 with levels of polymorphism above 40%. The mean levels of allelic variation over the nine *Per-2*, *Per-3*, and *Per-4* genes were 28%, 40%, and 21% for the A, B, and D genome loci, compared to mean values of 5%, 17%, and 5% over

the three genomes for the RFLP data. Interestingly, the B genome loci again appear to be more polymorphic than the other two.

Some previous analyses, using polyacrylamide and starch gel electrophoresis (Benito et al. 1980; Benito and Perez de la Vega 1979) have assumed that each grain peroxidase isozyme in hexaploid wheat is encoded by a single gene and, therefore, that some chromosomes carry more than one locus. The IEF analysis described above provides little evidence of whether the multiple isozyme patterns observed are due to modification of the products of single genes, which may include in vivo post-translation or artifacts of the separation system, or are the products of several closely linked genes. Similar complex isozyme patterns have been shown to be due both to complex genes, as found for α-amylase, *α-Amy-1* and *α-Amy-2* (Ainsworth et al. 1984), and apparently single genes, as found for β-amylase, *β-Amy-1* (Sharp et al. 1988). However, the segregational results do indicate that the PER-A4 isozymes are not encoded by dispersed genes on the same chromosome arm. Unfortunately, it has not yet been possible to undertake a similar analysis for all the *Per* loci, thus we have not been able to confirm or disprove the existence of separate linked loci on chromosomes *3B* and *3D*, as reported by Benito et al. (1980). Therefore the possibility, albeit unlikely, still exists that some of the *Per* loci described are, in fact, dispersed groups of genes.

Consideration of Fig. 4 and Table 2 raises a further problem related to assigning allelic designations by phenotype. The PER-3 system is extremely variable and, without segregational analyses involving all the isozymes, any classification is incomplete. We have been able to assign chromosomal control to only those isozymes in "CS", by nullisomic analysis, and to those isozymes that differ between pairs of varieties for which intervarietal chromosome substitution sets are available. Analysis of the remaining isozymes, assuming that they are controlled by the *Per-3* loci as seems most likely, will identify further alleles to those described above. This possibility exists, however, for all allelic designations, other than those described by DNA sequence data, because further differences may always be revealed by more precise or different methods of seperation.

A further difficulty can also be seen in the PER-3 analysis. With such a variable system of so many isozymes, it is possible that the products of two or more loci could have similar electrophoretic properties. A possible example of this can be seen with isozymes "6a" and "6c" in *T. macha* and "Timstein". Substitution analysis showed the two isozymes in the former to be controlled by *Per-B3* (Fig. 5A) and two similar isozymes in the latter to be controlled by *Per-A3* (Fig. 5B). However, we cannot rule out the possibility that they are indeed the same isozymes, and the different chromosome locations have resulted from a translocation between chromosome *3A* and *3B* in *T. macha* relative to the *T. aestivum* lines. For this reason these two isozymes were not accounted for in the allelic classication of "Timstein", *T. macha*, or "Timgalen", which had a similar phenotype and for which no intervarietal substitution lines exist.

It is likely, as was accomplished here with *Per-A4*, that the *Per-1*, *Per-2*, *Per-3*, and *Per-5* sets of genes will soon be incorporated into the emerging RFLP-based genetic maps of the homoeologous group 1, group 2, and group 3 chromosomes. As such, they will join the growing number of isozyme loci in wheat that will, for some time to come, provide inexpensive and more rapidly assayed markers than DNA probes.

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