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Inheritance and linkage relationships of peroxidase isoenzymes in apple

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Summary. Six anodal and two cathodal zones of peroxidase activity were observed in apple and designated PRX-I to PRX-VIII from the most anodal to the most cathodal on the basis of migrational distance. PRX phenotypes were found to be coded by at least eight genes (*PRX-1–8*); analyses of four (*PRX-2*, *PRX-3*, *PRX-4* and *PRX-7*) revealed 15 alleles including three null alleles. *PRX-2* and *PRX-3* were closely linked, and *PRX-1* appeared to be in the same linkage group. In addition, *PRX-4* and *PRX-5* were linked.

Key words: *Malus pumila* Mill – Peroxidase – Genes *PRX-1*, *PRX-2*, *PRX-3*, *PRX-4*, *PRX-5*, *PRX-6*, *PRX-7*, *PRX-8* – Linkage

Introduction

In apple, peroxidase (PRX, EC.1.11.1.7) activity and polymorphism has been found in seed embryos (Rychter and Lewak 1971), bark (Misic et al. 1980a; Wang et al. 1981) and in callus and cell suspension culture (Berger et al. 1985). Activity was not found in pollen (Veidenberg and Safonov 1968). Schaefer (1982) found different PRX patterns in roots and shoots of different ages. Cheng (1982) found that two bands of slow electrophoretic mobility were expressed in mature leaves but not in young unexpanded leaves.

Seven groups of bands, six anodal and one cathodal, have been described, and the inheritance of three anodal groups (C, D and E consisting of two, two, and three bands, respectively) was studied by Misic et al. (1980b). They concluded that at least seven closely linked polymorphic genes code for these bands with one active and one inactive (null) allele for each gene. Chevreau and Laurens (1987) described four groups of bands in the same area, A, B, C and D and suggested that they were coded by two genes, '*Per 1*' and '*Per 2*', each with 3 alleles, including one null allele. Bournival and Korban (1987) described a gene '*PER-2*' with 5 alleles coding for peroxidase activity in the same area. In order to clarify the genetics of peroxidase isoenzymes in apple the present study, based on controlled crosses between a wide range of cultivars and selections, was undertaken.

Materials and methods

Plant material

Progenies derived from crosses involves 45 apple cultivars and selections were studied. Various tissues were used in sample preparation including leaves, bark, flower buds, cotyledons, seed and roots; pollen did not show peroxidase activity.

Sample preparation

The extraction solution was 0.05 *M* Na-phosphate buffer pH 7.2 and 0.4 *M* sucrose. When fresh leaves and cotyledons were used, a ratio of 1 : 6 w/v of tissue to buffer was found to be satisfactory; with other tissues a ratio of 1 : 8 w/v was preferred. After homogenization with a mortar and pestle the homogenate was centrifuged for 30 min at 30,000 *g*. A sample of 30 µl supernatant gave the best resolution without losing any active bands.

Electrophoretic procedure

Samples were run on polyacrylamide slab gels (18 × 14 × 0.15 cm) prepared according to Davis (1964) with slight modifications. A "sample" gel was not used and *Tris*-glycine pH 8.8 (0.19 *M*) was incorporated into the "running" gel in place of *Tris*-HC1. Samples were run at constant voltage (100 V) until they passed into the "stacking" gel (approximately 30 min) and then at 150 V until they entered the "running" gel (approximate-

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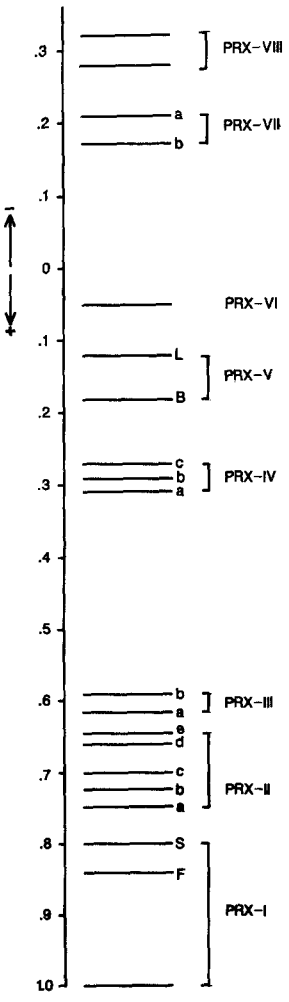

Fig. 1. Schematic representation showing the relative migration distance (*0* = start of "running" gel, *1.0* = fastest band in leaves) of PRX electrophoretic bands. Secondary bands are not shown. *PRX-V, L* band appearing in leaves, *PRX-V, B* band appearing in bark

ly 30 min more) when the voltage was increased to 350 V for 3 h. The same procedure was used for cathodal bands, but the polarity was reversed. After electrophoresis gels were stained for PRX using 3-amino-9-ethyl carbazole as substrate and 50 µl of 30% H_2O_2 in 100 ml staining solution (Hoyle 1978). Stained gels were washed with tap water and fixed in 7% acetic acid.

Results

Electrophoretic patterns

Six anodal and two cathodal zones of PRX activities, designated PRX-I to PRX-VIII from the most anodal to the most cathodal, were distinguished on the basis of their migrational distance and on the genetic studies presented below (Fig. 1). The relative distance (Rf value) of the bands was derived from the mean value of many "runs", calculated after assigning Rf = 1.0 to the fastest band and Rf = 0.0 to the start point of the "running" gel.

Table 1. Segregation for fast and slow bands in the PRX-I zone

Cross	Parental phenotypes	Progeny phenotypes	χ^2	*P*
F54 'Gloster 69' × 'Idared'	FS × FF	7FF:9FS	0.25	0.62
F55 'Gloster 69' × T30-24	FS × FF	11FF:9FS	0.20	0.66

Genetic control of PRX electrophoretic variants

Zone PRX-I. Depending on the organ examined, the most anodal group, PRX-I consisted of ten (bark) or more (leaves) bands migrating close to each other located from Rf = 0.80 to Rf = 1.00 (Fig. 1).

Genetic interpretation was difficult because young leaves, the main plant material used, showed low activity in this zone. Interpretation became even more difficult as a result of tissue-specific patterns and seasonal and developmental changes. Flower buds showed good activity, particularly during bud burst. Polymorphism could only be scored for the slower part of this zone, with S assigned to the slowest band and F to the faster adjacent band.

In two crosses, where clear bands were observed from bark extracts, the segregation (Table 1) followed that of a single locus, *PRX-1*, with 2 alleles *F* (coding the fast band) and *S* (coding the slow band).

Zone PRX-II. Five different band positions were observed, a, b, c, d and e from the most anodal to the most cathodal. Positions a and b each had a secondary band anodal to the major band. Position c had one, two or three secondary bands associated anodally to the major band, depending on the tissues studied. The first secondary c band migrated in the same position as the major b band, resulting in an intense band in that position when major bands of both b and c were present (Fig. 2). In this case, when leaves were used, two very closely migrating bands resulted in a distinct band anodal to the b band position. No secondary bands were observed for both positions d and e. When both parents showed two bands, with at least one different, four phenotypes were observed in progenies in a ratio approximately 1:1:1:1 (Table 2). When both parents showed the same two bands three phenotypes were observed in a ratio close to 1 : 2 : 1. In some cases the single band of one parent was only observed in about half of the progeny, suggesting the occurrence of a null allele. Amongst the 40 progenies 19 different combinations of bands were examined. These bands segregated according to the ratio expected for a single gene *PRX-2*, each band position being coded by a different allele *a*, *b*, *c*, *d* and *e* and additionally a null allele *n* for no activity. Homozygotes for the 5 active alleles exhibited activity in one of the five positions. Heterozygotes behaved in a codominant fashion, exhibit-

Table 2. Segregation for *PRX-2*

Family		Parental genotypes	Progeny genotypes	Expected ratios	χ^2	*df*	*P*
F814	('Cox' × A467-74)	bc × ce	12bc:9be:10cc:9ce	1:1:1:1	0.60	3	0.90
F12,17[a]	(T30-24 × 'Cox')	ce × bc	5bc:7be:9cc:7ce	1:1:1:1	1.14	3	0.76
F13,14[a]	(T32-2 × 'Cox')	ce × bc	5bc:9be:11cc:3ce	1:1:1:1	5.71	3	0.12
F16	('Cox' × T31-12)	bc × ce	3bc:3be:5cc:3ce	1:1:1:1	0.85	3	0.83
F36,49[a]	('Northern Spy' × 'Winter Majetin')	bc × ce	20bc:21be:33cc:19ce	1:1:1:1	5.60	3	0.13
F1,3[a]	(A463-70 × 'Cox')	bd × bc	11bb:18bc:8bd:13cd	1:1:1:1	4.24	3	0.24
F135	('Idared' × A679-12)	en × bc	21be:19bn:20ce:22cn	1:1:1:1	0.24	3	0.97
F2	(A463-70 × 'Granny Smith')	bd × de	9bd:6de:6dd:5be	1:1:1:1	1.30	3	0.73
F115	('Kent' × 'Fiesta')	cd × be	5bc:6bd:5ce:11de	1:1:1:1	3.67	3	0.30
F106	(T30-9 × T31-12)	bn × ce	12bc:14cn:2be:11en	1:1:1:1	8.69	3	0.04*
F18	('Cox' × 'Fiesta')	bc × be	5bb:8bc:4be:5ce	1:1:1:1	1.63	3	0.65
F70	(A172-2 × A814-137)	ab × ce	4ac:4ae:7bc:1be	1:1:1:1	4.50	3	0.21
F37	('Granny Smith' × 'Kent')	de × cd	8cd:15ce:15dd:7de	1:1:1:1	5.04	3	0.17
F27	('Delprim' × 'Katy')	bc × bc	4bb:14bc:10cc	1:2:1	2.57	2	0.28
F63	('Cox' S. F. clones selfed)	bc × bc	9bb:21bc:8cc	1:2:1	0.47	2	0.79
F98	(A722-6 × 'Cox')	bc × bc	2bb:6bc:4cc	1:2:1	0.67	2	0.72
F99	(A723-14 × 'Jester')	bc × bc	6bb:7bc:6cc	1:2:1	1.31	2	0.52
F100	(A723-5 × 'Jester')	bc × bc	9bb:16bc:9cc	1:2:1	0.12	2	0.94
F101	(A723-6 × 'Jester')	bc × bc	9bb:18bc:11cc	1:2:1	0.31	2	0.85
F103	('Northern Spy' × 'Cox')	bc × bc	3bb:6bc:6cc	1:2:1	1.80	2	0.41
F5,6[a]	('Idared' × T31-12)	en × ce	13ce:29(ee+en):14cn	1:2:1	0.11	2	0.95
F11,21[a]	('Idared' × 'Fiesta')	en × be	17be:20(ee+en):8bn	1:2:1	4.11	2	0.14
F48	('Jonathan' × 'Idared')	ce × en	16ce:21(ee+en):9cn	1:2:1	2.48	2	0.27
F30	('Idared' × 'Golden Hornet')	en × cc	4ce:10cn	1:1	2.57	1	0.11
F34	('Idared' × 'Spartan')	en × cc	23ce:23cn	1:1	0.00	1	1.00
F54	('Gloster 69' × 'Idared')	cc × en	8ce:11cn	1:1	0.47	1	0.49
F41	('Cox' × 'Baskatong')	bc × cc	22bc:34cc	1:1	2.57	1	0.11
F23	('Vista Bella' × 'Katy')	cc × bc	8bc:13cc	1:1	1.19	1	0.27
F132	('Katy' × 'White Angel')	bc × cc	42bc:29cc	1:1	2.38	1	0.12
F109	('Cox' × 'Greensleeves')	bc × cc	13bc:11cc	1:1	0.17	1	0.68
F72	(604 × A853-1)	ce × cc	6cc:12ce	1:1	2.00	1	0.16
F69	(3759 × 'Baskatong')	ce × cc	8cc:10ce	1:1	0.22	1	0.64
F93	('Jonathan' × A849-7)	ce × cc	16cc:15ce	1:1	0.03	1	0.86
F55	('Gloster 69' × T30-24)	cc × ce	12cc:8ce	1:1	0.80	1	0.37
F32	('Fiesta' × 'Spartan')	be × cc	6bc:7ce	1:1	0.08	1	0.78
F46	('Discovery' × 'Red Jade')	bb × cd	13bc:13bd	1:1	0.00	1	1.00
F9,10[a]	('Golden Delicious' × A463-70)	cc × bd	25bc:28cd	1:1	0.17	1	0.67
F63	('Spencer's Seedless' × 'George Carpenter')	cc × bd	46bc:50cd	1:1	0.17	1	0.89
F71	(A140-7 × A172-2)	cc × ab	12ac:19bc	1:1	1.58	1	0.21
F562	('Granny Smith' × SA219-21)	de × cc	15cd:19ce	1:1	0.47	1	0.49

* $P<0.05$

[a] Pooled data from reciprocal crosses

ing bands of both alleles unless one was the null allele. No 'hybrid' bands were observed, which suggests a monomeric structure for this enzyme.

In seven F_2 type crosses the expected 1 : 2 : 1 ratio was observed. No heterogeneity was detected amongst these three-class segregating progenies ($\chi^2 = 5.34$, 12 *df*) or in five crosses with the 1:1:1:1 expected ratio (bc × ce and reciprocals) ($\chi^2 = 5.45$, 12 *df*). These latter progenies showed consistently a surplus of *cc* seedlings and a deficiency of *ce* seedlings, that was significant ($P = 0.04$). One further progeny (F106: *bn* × *ce*) showed a significant deviation from the expected ratio ($P = 0.04$). Selective fertilization may have been responsible. No difference was detected between reciprocal crosses. Although there was insufficient genetic evidence to identify further alleles, slight but constantly differing migration distances of some bands suggest that more alleles might be involved.

In progeny F54 ('Gloster 69' *PRX-2cc* × 'Idared' *PRX-2en*) 8 seedlings showed band c with two secondary bands and 11 band c with one secondary band in bark

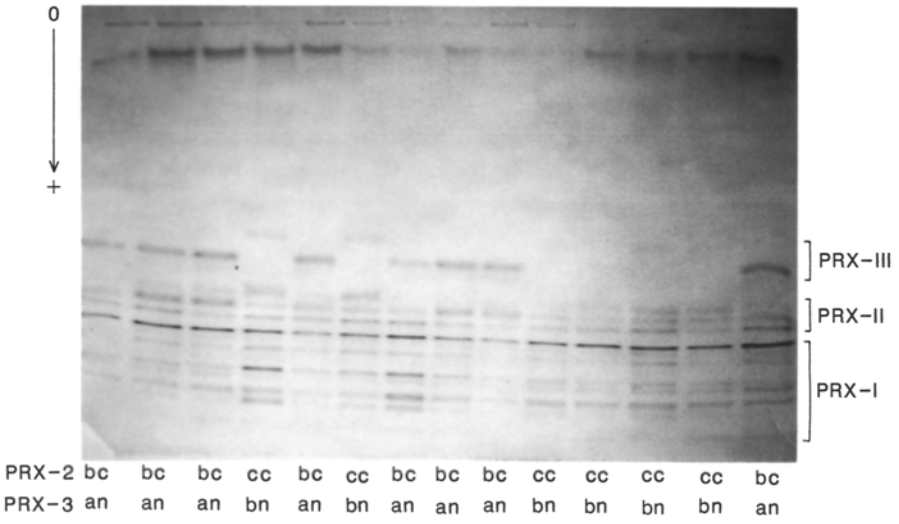

Fig. 2. Peroxidase zymograms from lyophilysed leaves of progeny from F109: 'Cox' (*PRX-2bc/3ab*) × 'Greensleeves' (*PRX-2cc/3nn*) showing segregation of alleles *PRX-2b, c* and *PRX-3a, b, n*

Table 3. Segregation for *PRX-3*

Family		Parental genotypes	Progeny genotypes	Expected ratio	χ^2	*df*	*P*
F140	('Glengyle Red' × 3762)	an × bn	9ab:13an:11bn:13nn	1:1:1:1	0.96	3	0.81
F36, 49[a]	('Northern Spy' × 'Winter Majetin')	ab × bn	21ab:52(bb + bn):20an	1:2:1	1.32	2	0.52
F54	('Gloster 69' × 'Idared')	bn × ab	5ab:8(bb + bn):6an	1:2:1	0.58	2	0.75
F11, 21[a]	('Idared' × 'Fiesta')	ab × ab	8aa:24ab:14bb	1:2:1	1.65	2	0.44
F18	('Cox' × 'Fiesta')	ab × ab	5aa:12ab:5bb	1:2:1	0.18	2	0.91
F37	('Granny Smith' × 'Kent')	ab × ab	15aa:16ab:14bb	1:2:1	3.80	2	0.15
F63	('Cox S. F.' selfed)	ab × ab	9aa:21ab:8bb	1:2:1	0.47	2	0.79
F103	('Northern Spy' × 'Cox')	ab × ab	3aa:6ab:6bb	1:2:1	1.80	2	0.40
F115	('Kent' × 'Fiesta')	ab × ab	6aa:16ab:5bb	1:2:1	1.00	2	0.61
F98	(A722-6 × 'Cox')	an × ab	2ab:6(aa + an):4bn	1:2:1	0.66	2	0.72
F100	(A723-5 × 'Jester')	ab × an	9ab:16(aa + an):9bn	1:2:1	0.12	2	0.94
F101	(A723-6 × 'Jester')	ab × an	13ab:14(aa + an):11bn	1:2:1	2.84	2	0.25
F135	('Idared' × A679-12)	ab × an	20ab:41(aa + an):21bn	1:2:1	0.02	2	0.99
F99	(A723-14 × 'Jester')	ab × an	3ab:10(aa + an):6bn	1:2:1	1.00	2	0.60
F24	('Golden Delicious' × 'Golden Hornet')	bn × bn	17(nn + bn):10nn	3:1	2.09	1	0.15
F25	('Golden 69' × 'Golden Hornet')	bn × bn	21(bb + bn):6nn	3:1	0.11	1	0.74
F27	('Delprim' × 'Katy')	an × an	18(aa + an):10nn	3:1	1.71	1	0.19
F1, 3[a]	(A463-70 × 'Cox')	aa × ab	19aa:31ab	1:1	2.88	1	0.09
F2	(A463-70 × 'Granny Smith')	aa × ab	15aa:12ab	1:1	0.33	1	0.56
F9, 10[a]	('Golden Delicious' × A463-70)	bn × aa	32ab:21an	1:1	2.28	1	0.13
F71	(A140-7 × A172-2)	bn × aa	14ab:17an	1:1	0.29	1	0.59
F23	('Vista Bella' × 'Katy')	nn × bn	8bn:13nn	1:1	1.19	1	0.27
F5, 6[a]	('Idared' × T31-12)	ab × bb	26ab:30bb	1:1	0.28	1	0.60
F12, 17[a]	(T30-24 × 'Cox')	bb × ab	12ab:16bb	1:1	0.57	1	0.45
F13, 14[a]	(T32-2 × 'Cox')	bb × ab	14ab:14bb	1:1	0.00	1	1.00
F16	('Cox' × T31-12)	ab × bb	6ab:8bb	1:1	0.28	1	0.59
F48	('Jonathan' × 'Idared')	bb × ab	21ab:25bb	1:1	0.35	1	0.55
F814	('Cox' × A467-74)	ab × bb	21ab:16bb	1:1	0.68	1	0.41
F34	('Idared' × 'Spartan')	ab × nn	23an:23bn	1:1	0.00	1	1.00
F41	('Cox' × 'Baskatong')	ab × nn	22an:34bn	1:1	2.57	1	0.11
F32	('Fiesta' × 'Spartan')	ab × nn	6an:7bn	1:1	0.08	1	0.78
F46	('Discovery' × 'Red Jade')	ab × nn	13an:13bn	1:1	0.00	1	1.00
F562	('Granny Smith' × SA219-21)	ab × nn	15an:20bn	1:1	0.71	1	0.40

[a] Pooled data from reciprocal crosses

extracts. Until the inheritance of bands in this zone is further clarified, *c* must be considered to code for both patterns.

Interaction between the *b* and *c* alleles was observed. When both alleles were present the b band was more intense than the c band (Fig. 2).

Zone PRX-III. This consists of two band positions, a and b, both having a major band with a secondary fainter band in an anodal position. Segregation of the two major band positions was recorded in 33 progenies, results (Table 3) suggest that a single gene, *PRX-3* is responsible for activity in this zone, with the alleles *a* and *b* corresponding to band positions a and b, respectively. In addition a null allele *n* occurred in this zone (Fig. 2); as a result seedlings without PRX-III activity were observed in some progenies. In progenies where the exact genotype of the seedlings could not be determined because of null alleles, the two genotypes with a similar phenotype were combined (F36, F49, F98, F99, F100, F101, F135). In three progenies (F24, 25, 27) where both parents carried a null allele the segregation of seedlings showing PRX-III activity agreed well with the expected 3 : 1 ratio.

Zone PRX-IV. Since all the cultivars involved are monomorphic in this zone, which consists of a sixplet with one major band and five secondary bands of decreasing intensity at a uniform distance from each other and distal to the major band (Rf 0.29, 0.31, 0.33, 0.35, 0.37, 0.39), no segregation was observed in most crosses. However, two additional band positions were found amongst *Malus* species (each consisted of a sixplet with the major bands migrating at Rf 0.31 and 0.27). The positions of the three major bands were designated a, b and c (Fig. 1). The segregation of four crosses involving *Malus* sp. derivatives are shown in Table 4. These results suggest that a single gene, *PRX-4*, is responsible for activity in this zone, with three alleles *a*, *b* and *c* corresponding to the a, b and c band positions respectively.

There were considerable differences between the tissues examined. Bark and roots nearly always showed the characteristic sixplets. Bark from 2-year-old shoots gave more intensive bands than bark from 1-year-old shoots. Cotyledons showed only the major bands, while young leaves showed low activity with only very faint major bands.

Zone PRX-V. This consists of one slowly migrating band different in leaves (Rf 1.12) and bark (Rf 1.18). With leaves only old ones showed activity, and resolution, which was good, was improved by using lyophilized leaves and 4-chloro-1-naphthal as the H^+ donor in the staining solution. Nearly all of the cultivars examined were monomorphic in this zone, hence it was difficult to determine whether one or more loci were involved. No activity was observed in M.15, *Malus floribunda* and *M.* × *robusta* bark extracts (Fig. 3), suggesting the presence of null alleles in these cases. However, flower buds of *M. floribunda* clone J showed a single band in this zone.

Zone PRX-VI. This consists of a single, slowly migrating (Rf 0.05) band observed in bark and flower buds but not in leaves. Seasonal changes were observed, with activity being the greatest in late autumn and winter. Hanker-Yates reagent (Hanker et al. 1977) used as the hydrogen donor gave the best staining in this zone. All the cultivars examined showed this band, however most of the *Malus* species and some derivatives lacked PRX-VI activity. Segregating progenies were not available for genetic studies in this zone. Misic et al. (1980a) included this zone with PRX-V and described them as cluster A.

Zones PRX-VII and PRX-VIII. The patterns in these cathodal zones were not always sufficiently clear to score accurately. The zone closest to the origin, PRX-VII, showed two band positions, a and b (Rf 0.21 and 0.17, respectively). Zone PRX-VIII moved faster at Rf 0.28–0.32, but activity was consistent.

Results from seedlings of segregating progenies suggest (Table 5) the existence of a gene *PRX-7* with two alleles *a* and *b* coding bands a and b, respectively. The large deviation observed in progenies F36 and F49 suggest a further locus may be involved in some cases. The occurrence of a null allele *n* explains the segregation observed in progeny F140.

Table 4. Segregation for *PRX-4*

Family		Parental genotypes	Progeny genotypes	Expected ratio	χ^2	*df*	*P*
F104	(A721-19 × 'Cox')	ab × bb	7ab:7bb	1:1	0.00	1	1.00
F135	('Idared' × A679-12)	bb × ab	36ab:31bb	1:1	0.37	1	0.54
F140	('Glengyle Red' × 3762)	bb × bc	19bb:14bc	1:1	0.76	1	0.38
F814	('Cox' × A467-74)	bb × bc	21bb:19bc	1:1	0.10	1	0.75

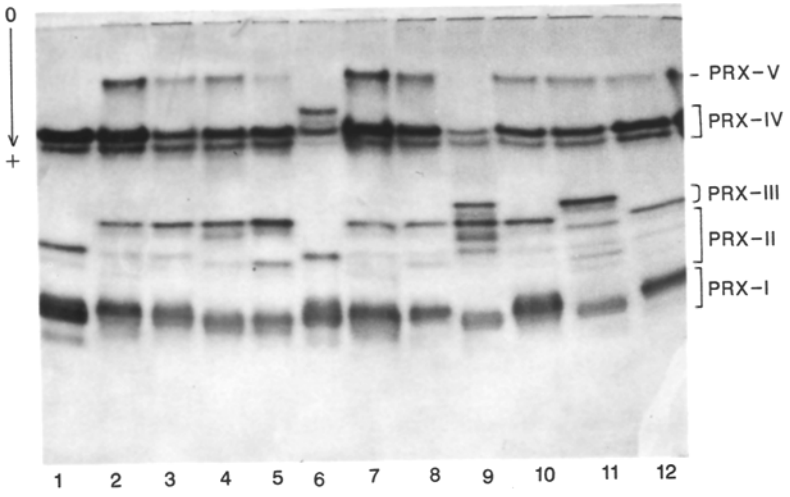

Fig. 3. Peroxidase zymograms in apple shoot bark: *1 M. floribunda*, *2* M.7, *3* M.13, *4* M.19, *5* MM.106, *6 M.* × *robusta*, *7* M.7, *8* M.6, *9* M.15, *10* M.4, *11* MM.112, *12* M.9 EMLA

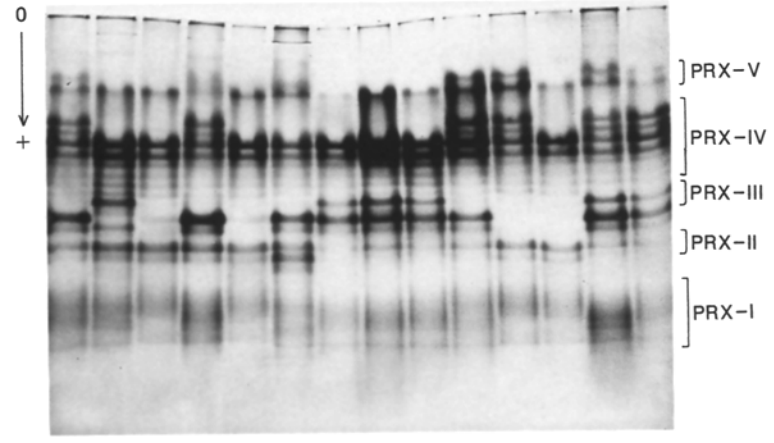

Fig. 4. Peroxidase zymograms in bark of apple progeny F140 ('Glengyle Red' × 3762). Note the association of segregation of the PRX-IV and PRX-V zone except for the sixth seedling from the left

Table 5. Segregation for *PRX-7*

Family		Parental genotypes	Progeny genotypes	Expected ratio	χ^2	*df*	*P*
F101	(A723-6 × 'Jester')	bb × ab	14ab:13bb	1:1	0.04	1	0.85
F135	('Idared' × A679-12)	bb × ab	10ab:13bb	1:1	0.39	1	0.53
F140	('Glengyle Red' × 3762)	aa × bn	26an:20ab	1:1	0.78	1	0.37
F36	('N. Spy' × 'W. Majetin')	bb × ab	29ab:17bb	1:1	3.13	1	0.08
F49	('W. Majetin' × 'N. Spy')	ab × bb	18ab:5bb	1:1	7.34	1	0.007**

$P<0.01$

Linkage between PRX loci

PRX-2 and *PRX-3* appeared to be very closely linked. However, the presence of null alleles greatly reduced the effectiveness of measuring recombination frequencies. In only 4 of the 25 progenies studied could every recombinant class have been detected even if they had all been present. In the remaining 21 progenies it would have been possible to detect only 25–75% of the recombinants. Nearly complete agreement between the segregations of both genes was observed. Only two recombinants were detected in a total of 1,041 plants. In terms of the number of tested gametes (1,155) the recombination frequency was 0.0017.

Of the 18 possible combinations of linkage between the 6 alleles of *PRX-2* and the 3 alleles of *PRX-3* 11 were confirmed amongst 71 varieties used as parents. Certain alleles seemed to be linked preferentially. *PRX-2b* was

linked with *PRX-3a* in 15 varieties and only once with *PRX-3b*. *PRX-2c* was linked with *PRX-3b* in 17 varieties, with *PRX-3n* in 18 varieties and never with *PRX-3a*.

PRX-1 seems to be linked with *PRX-2* and *PRX-3*. In progeny F54 ('Gloster 69' × 'Idared') where 'Gloster 69' segregates for *PRX-1* (FS) and *PRX-3* (*bn*), only three types of gametes were recognized as being derived from 'Gloster 69': 7 *PRX-1F/PRX-3b*, 8 *PRX-1S/PRX-3n* and 1 *PRX-1S/PRX-3b* which probably resulted from crossing over between *PRX-1* and *PRX-3*. Similarly, in progeny F55 ('Gloster 69' × T30/24) four types of gametes were recognized from 'Gloster 69': 10 *PRX-1F/PRX-3b*, 8 *PRX-1S/PRX-3n*, 1 *PRX-1F/PRX-3n* and 1 *PRX-1S/PRX-3b*, these latter two being recombinants between *PRX-1* and *PRX-3*.

Finally, it seems that *PRX-4* might be closely linked with *PRX-5*. In the progeny F140 ('Glengyle Red' × 3762) segregation occurred at both the *PRX-4* and *PRX-5* loci. All except 1 of the 33 seedlings examined which had a slow PRX-V band also had the allelic band c in the PRX-IV zone (Fig. 4).

Discussion

The genetic studies show that the PRX phenotypes described are coded by at least eight genes (*PRX-1-8*); analyses of four genes (*PRX-2*, *PRX-3*, *PRX-4* and *PRX-7*) show 15 alleles, including three null alleles. The genetic interpretation of the PRX-II and PRX-III zones is more precise than that in previous reports (Misic et al. 1980b; Bournival and Korban 1987; Chevreau and Laurens 1987). Since no more than four band positions were present each time for both zones, it was deduced that not more than two genes determined their activity. This hypothesis is more plausible than that proposed by Misic et al. (1980b) where at least seven genes were proposed to account for activity in these zones, each band being determined by a different gene. Although no more than four different phenotypic groups were observed, in the *PRX-II* and *PRX-III* zones, amongst the many progenies examined, the recovery of two recombinants indicates that two very closely linked genes are involved.

These findings firmly substantiate the suggestions of Chevreau and Laurens (1987) for the two linked genes *Per 1* and *Per 2* which were based on limited data. Three new alleles are proposed for *PRX-2*, and the conclusions on *PRX-2/PRX-3* linkage are based on 25 segregating progenies. In view of this close linkage an origin by tandem duplication resulting from errors in pairing and crossing over can be proposed for *PRX-2* and *PRX-3*. There is a high degree of apparent preferential linkage between alleles of the two genes and an almost complete absence of some combinations. Although as most possible combinations occur, it must be supposed that two distinct genes (*PRX-2* and *PRX-3*) are involved. This phenomenon of linked mimics has also been reported in other crops (Kahler and Allard 1970; Tanksley and Rick 1980).

The clarification of the genetics of the PRX-II, PRX-III, and PRX-VI anodal zones and the PRX-VII cathodal zone identifies a series of distinct genetic markers for breeding studies. In particular *PRX-2* with six alleles has the potential to provide sufficient variation to facilitate variety identification on the basis of genotype. It remains to be seen whether or not the apparent preferential pairing between alleles of this gene and those of *PRX-3* results from an association between alleles of these genes and alleles of other genes, which determine important agronomic characters, and whether they can be used as early selection markers in breeding programmes. Tissue-specific differences and developmental changes could be used to attribute certain physiological roles to certain allelic bands. The presence of activity in the slow zones PRX-V and PRX-VI when old leaves were used, and the almost complete absence of activity using young leaves was also reported by Cheng (1982). However, a functional role cannot be assigned. Liu's (1973) techniques, which can be used to measure the activity of individual bands, might prove useful to follow changes in peroxidase levels that might be correlated with developmental processes.

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