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Genetics of the Peroxidase Isoenzymes in *Petunia*

Part 4. Location and Developmental Expression of the Structural Gene *prxC*

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Summary. By starch gel electrophoresis three mobility variants of a cathodic moving doublet of bands, encoded by the structural gene *prxC*, were detected in all organs of flowering petunias. In root tissue two of the variants showed a lower electrophoretic mobility than in other organs. During development of flower buds the PRXc enzymes showed an increase in mobility. The gene *prxC* was located on chromosome IV by showing linkage to the genes *An3* and *Dwl*, by trisomic segregation, and by the construction of triply heterozygous trisomics IV. The gene order on chromosome IV is *Bl-An3/Dwl-prxC*. It was concluded that the temporal programming difference in the expression of the alleles *prxC2* and *prxC3* is caused by internal site mutation. Analysis of progeny obtained by crossing of lines to the trisomic IV with genotype *prxC1/C1/C2* showed differential expression of the two *prxC1* alleles of the trisomic IV.

Key words: *Petunia* – Peroxidase – Developmental expression – Gene localization

Introduction

Higher plants contain a considerable number of peroxidase isoenzymes (Scandalios 1974). The high number of peroxidases in plants is the result of the action of several structural genes (Pai et al. 1973; Felder 1976; Benito and Perez de la Vega 1979; Benito et al. 1980; Tanksley and Rick 1980; van den Berg and Wijsman 1981, 1982a, b; Endo 1981; Quiros and Morgan 1981; Garcia et al. 1982).

In *Petunia*, three structural genes encoding the major peroxidases present in leaves could be distinguished by their independent inheritance. The genes *prxA* and *prxB*, encoding the anodic peroxidases have been located on chromosome III and I, respectively (van den Berg and Wijsman 1982a, b). Here we report the location of the gene *prxC*, coding for the major cathodic isoenzymes present in leaves.

In addition to the involvement of several structural genes, the phenomenon that one allele encodes more than one peroxidase contributes to the complexity of the peroxidase system (Denna and Alexander 1975; Hirano and Naganuma 1971; Rick et al. 1979; van den Berg and Wijsman 1981, 1982b). In the case of *prxA*, (the gene coding for the fast-moving anodic group of peroxidases, the PRXa complex), three lines of evidence showed that four bands are encoded by one *prxA* allele. First, electrophoretic variation of the PRXa complex as a unit suggests that the mobility shift is caused by one mutation. Second, the complex is inherited in a monogenic fashion. Third, an altered expression caused by a cis-acting temporal mutation affects the activity of all the enzymes of the complex (van den Berg and Wijsman 1981, 1982b). Below, similar lines of evidence are presented for the gene *prxC* encoding cathodic moving doublets of bands.

Materials and Methods

Plant Material

The following inbred lines were used: *P. axillaris:* S1, S2, S8; *P. inflata:* S6, S9, S10; *P. parodii:* S7 (the origin of the *P. axillaris*, *P. inflata* and *P. parodii* inbred lines has been given earlier, van den Berg and Wijsman 1982a); *P. hybrida:* W4 (derived from the cultivar "Pendula Cyanea"), A4 (derived from the cultivars "Fire Chief" and "Pendula Cyanea"), W28 (derived from the cultivar "Roter Vogel"), and M57, R12, R74, R80, W39 (lines of recombinant descent). For the construction of triply heterozygous trisomics and the trisomic segregation, the trisomic IV B3014 was used, which was kindly provided by Dr. Maizonnier and Dr. Cornu (I.N.R.A., Dijon, France). Plants of *P. integrifolia* and *P. inflata* were grown from seeds collected in South-America and kindly sent by Dr. T. M. Pedersen.

Electrophoretic Analysis of the Peroxidase Isoenzymes

Sample preparation, electrophoretic separation of the peroxidase isoenzyme using gel system II, staining, and densitrometric scanning of the zymograms were carried out essentially

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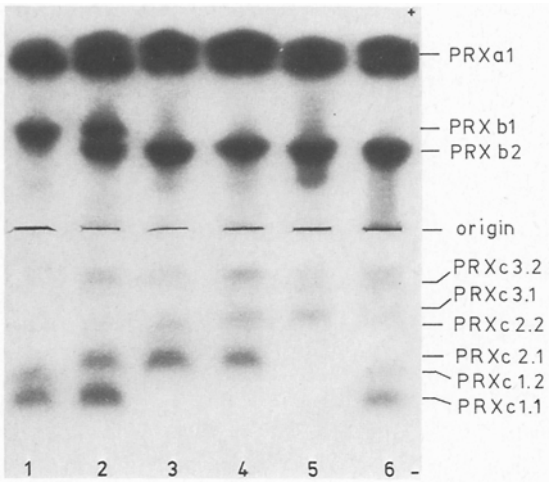

Fig. 1. System II starch gel showing the three variants of the PRXc doublet and the anodic peroxidases from mature leaves of young flowering plants. (1) *P. axillaris* S1, *prxC1/C1;* (2) F1 (*P. axillaris* S1×*P. axillaris* S2), *prxC1/C2*; (3) *P. axillaris* S2, *prxC2/C2*; (4) F1 (*P. hybrida* W4×*P. hybrida* W28), *prxC2/C3*; (5) *P. axillaris* S8, *prxC3/C3*; (6) F1 (*P. axillaris* S1×*P. hybrida* W4), *prxC1/C3*

as described previously (van den Berg and Wijsman 1981, 1982a). The electrophoretic procedure was modified in that the electrode buffers were reused several times, and that the pH of the gel buffer was changed to 8.2 with NaOH for better separation of the PRXc isoenzymes. In addition, gel system III, consisting of a gel containing 12.5% starch, 9 mM Tris, 3 mM citric acid, pH 7.0, and electrode buffer containing 130 mM Tris, 43 mM citric acid, pH 7.0 (essentially according to Siciliano and Shaw 1976) was used.

Nomenclature of the Peroxidase Genes and Enzymes

As described elsewhere (van den Berg and Wijsman 1981) the structural gene encoding the cathodic moving doublets is termed *prxC*. Alleles and enzymes are numbered; when more than one band is encoded by an allele, an additional number is given, e.g., the allele *prxC1* encodes the isoenzymes PRXc1.1 and PRXc1.2. The definition of internal and external site mutation as given previously is used here (van den Berg and Wijsman 1982a, b).

Determination of Genotypes for the Genes An3, B1, and Dw1

Genotypes for *An3,* a gene essential for the synthesis of flavonols and anthocyanins, could be determined by the flower colour: the *an3an3* genotype results in a white flower (Wiering 1974). The gene *Dw1* is, when homozygous and recessive, responsible for a dwarf phenotype of the plant, and the gene *B1* (blind) prevents, when homozygous and recessive, the formation of flower petals (Wiering et al. in preparation).

Characterization of Trisomics IV

Trisomics IV could be recognized from their typical flower morphology (short corolla, exerted pistil and anthers) as described by Maizonnier (1976). In addition, chromosome counts were routinely made according to de Jong and de Bock (1978).

Results

Electrophoretic Analysis of the PRXc Enzymes

Two variants of a cathodic moving doublet, encoded by the alleles *prxC1* and *prxC2,* have been reported previously (van den Berg and Wijsman 1981). The variation for *prxC* and the doublet phenotype could only be detected with the use of gel system II.

Among *P. hybrida* inbred lines a third allele was found with system II which codes for a PRXc doublet that moves slower than the PRXc1 and PRXc2 doublet (Fig. 1). Also, this variant could not be detected with the use of gel system I. However, in gel system II the genotypes *prxC2/C2* and *prxC2/C3* could not be separated properly. This is because the PRXc2.2 enzyme has nearly the same mobility as the PRXc3.1 enzyme, whereas the PRXc3.2 enzyme is hard to detect in young tissue. In older tissue other cathodic moving enzymes interfere with scoring the PRXc3.2 enzyme. Since the *prxC2* and *prxC3* alleles are more frequent among *P. hybrida* inbred lines than the *prxC1* allele, gel system III was used in addition to gel system II. In gel system III the bands encoded by alleles *prxC1* and *prxC3* have a similar mobility, but the PRXc2 enzymes have a lower mobility (Fig. 2). The peroxidases that have a slightly lower mobility than the PRXc3.2 enzyme (system II, Figs. 1, 3, 6, 7) were not detected in gel system III. We assume that they are not encoded by the gene *prxC*.

Electrophoretic Variation of the Peroxidase c Isoenzymes

In addition to the variants encoded by the alleles *prxC1* and *prxC2,* a third variant, with a lower electrophoretic mobility than the former two, was found among *P. hybrida* inbred lines (Fig. 1). Backcross and F2 segregation for *prxC* showed that the third variant, the PRXc3 doublet, is also encoded by the gene *prxC* (Fig. 2).

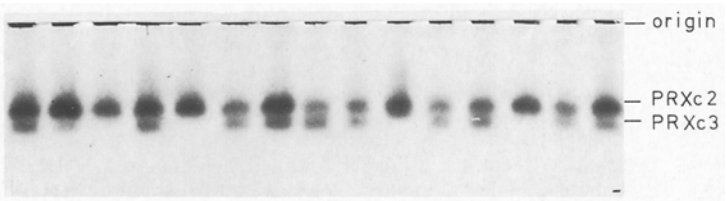

Fig. 2. System III starch gel showing segregation for prxC among the progeny of B1 (W4×W28)×W28. The segregation found was 82:68 for *prxC2/C2* and *prxC2/C3,* respectively ($X^2_{1:1}=1.31$; $P=0.60$). Samples were corolla tubes of young plants

The three PRXc variants were also detected in *Petunia* species related to *P. hybrida*. The *P. axillaris* inbred lines S1, S2 and S8 are homozygous for *prxC1*, *prxC2* and *prxC3*, respectively. The *P. inflata* inbred lines were originally heterozygous containing the alleles *prxC1* and *prxC2;* now, the lines S6 and S9 are homozygous for *prxC1*. In plants of *P. integrifolia* and *P. inflata*, germinated from seeds collected in South-America, the alleles *prxC1* and *prxC2* were found. Finally, line S7, *P. parodii*, contains the allele *prxC2*.

In root tissue an organ specific variation was found. The PRXc doublets of root-tissue show a slightly lower electrophoretic mobility than the doublets of other organs. This was shown for the PRXc1 and PRXc2 doublets (Fig. 3).

In flower buds three bud specific cathodic moving bands were detected (Fig. 4). These bands could not be detected in mature flowers or other parts of the plants. The fact that variation of these bands goes hand in hand with variation of the PRXc doublets suggests that the minor bands are also encoded by the gene *prxC*.

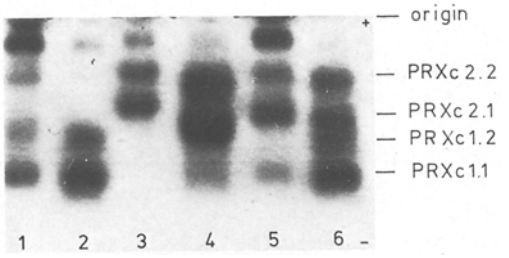

Fig. 3. System II starch gel showing the lower electrophoretic mobility of PRXc doublets of root tissue (1, 3, 5), when compared to the mobility of the doublets of corolla tubes (2, 4, 6). Samples were taken from full grown flowering plants. (1), (2), *P. axillaris* S1, *prxC1/C1*; (3), (4), *P. axillaris* S2, *prxC2/C2*; (5), (6), F1 (*P. axillaris* S1 × *P. axillaris* S2), *prxC1/C2*

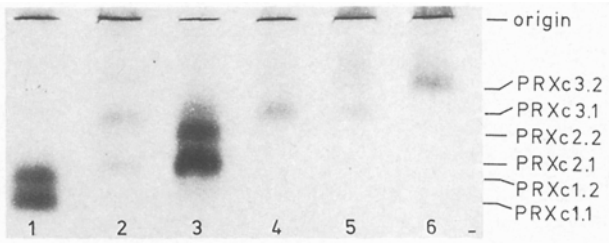

Fig. 4. System II starch gel showing peroxidase isoenzymes of corolla tubes (1, 3, 5) and buds (2, 4, 6) of young plants. (1), (3), and (5) show the PRXc doublets; (2), (4), and (6) show the bud specific bands. (1), (2), *P. axillaris* S1, *prxC1/C1*; (3), (4), *P. axillaris* S2, *prxC2/C2*; (5), (6), *P. axillaris* S8, *prxC3/C3*. Note that (2) shows two bud specific bands with a lower mobility than the PRXc1 doublet

Developmental Expression of the Gene prxC

The PRXc enzymes are present in every part of *Petunia*. Just as is the case with the PRXa and PRXb enzymes, the activity of the PRXc enzymes increases during development of organs and plant. In leaf tissue of young flowering plants the PRXc enzymes have a much lower activity than the PRXa and PRXb enzymes (Fig. 1). However, in general, the PRXc enzymes in the corolla tube have a higher activity (Figs. 4, 5, 7).

In young leaves of a young flowering plant (Fig. 2, van den Berg and Wijsman 1981) the faster moving enzyme of the PRXc doublets has a higher activity, but during development the relative activity of the slower moving one increases. In old leaves both bands have a similar activity.

Analysis of a series of flower buds of different ages revealed that the slower moving band appears first and increases in mobility during development. Just before flower opening the faster moving band of the doublet, appears and its activity increases dramatically during the opening of the flower, in the tube even more than in the limb. During further development of the corolla the bands of the doublet reach a similar activity in the tube, but in the limb both bands disappear, the slower moving one first (Fig. 5).

In plants heterozygous for *prxC* the slower band of the doublets can barely be detected in mature leaves of plants at the onset of flowering, but in plants homozygous for *prxC* they can be easily detected. This was not only found for homozygous inbred lines on the one hand and heterozygous F1 hybrids between inbred lines on the other, but also in plants from B1 and F2 progenies showing segregation for *prxC*.

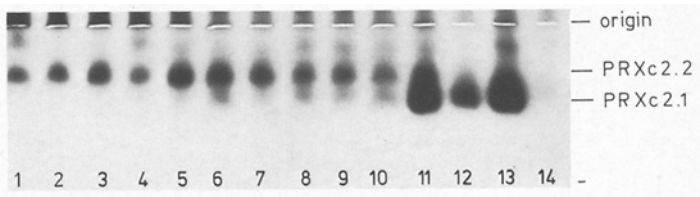

Fig. 5. System II starch gel showing the developmental expression of the cathodic peroxidases in flower tissue of *P. axillaris* S2. (1)–(10), young to old buds; (11), (12), corolla tube and limb, respectively, of a mature flower; (13), (14), corolla tube and limb, respectively, of an old flower

Location of prxC by Linkage Analysis

From the analysis of the progenies F2 (W4 × W39) and B1 [(R74 × R12) × R12], linkage of *prxC* to genes *An3* and *Dw1*, was found (Tables 1 and 2).

Genes *An3* and *Dw1* have been located on chromosome IV by Wiering et al. (in preparation), and are highly linked to one another. The genetic distances

Table 1. Segregation for the genes *prxC* and *An3* showing linkage

Cross F2 (W4×W39)
Genotype $\frac{prxC2,\ an3}{prxC3,\ An3} \times \frac{prxC2,\ an3}{prxC3,\ An3}$

Genotypes and number of progeny			
	prxC2/C2	*prxC2/C3*	*prxC3/C3*
An3–	21	77	35
an3an3	47	32	0

Segregation chi square tests				
prxC	68 : 109 : 35	$\chi^2_{1:2:1} = 10.44$	(df=2)	P=0.005
An3	133 : 79	$\chi^2_{3:1} = 17.0$	(df=1)	$P < 10^{-3}$

Linkage chi square test
$\chi^2_{3\times2} = 53.22$ (df=2) $P < 10^{-3}$

An3– stands for the heterozygous and homozygous dominant genotypes

Table 2. Segregation for the genes *prxC* and *Dw1* showing linkage

Cross B1 (R74×R12)×R12
Genotype $\frac{prxC2,\ Dw1}{prxC3,\ dw1} \times \frac{prxC3,\ dw1}{prxC3,\ dw1}$

Genotypes and number of progeny		
	prxC2/C3	*prxC3/C3*
Dwdw	141	45
dwdw	30	88

Segregation chi square tests				
prxC	171 : 133	$\chi^2_{1:1} = 4.75$	(df=1)	P=0.03
Dw1	186 : 118	$\chi^2_{1:1} = 15.21$	(df=1)	$P < 10^{-3}$

Linkage chi square test
$\chi^2_{2\times2} = 74.5$ (df=1) $P < 10^{-3}$

Table 3. Segregation for the genes *prxC* and *B1* showing independent inheritance

Cross F2 (S1×R51)
Genotype $\frac{prxC1,\ B1}{prxC2,\ b1} \times \frac{prxC1,\ B1}{prxC2,\ b1}$

Genotypes and number of progeny			
	prxC1/C1	*prxC1/C2*	*prxC2/C2*
B1–	31	78	39
b1b1	12	26	23

Segregation chi square tests				
prxC	43 : 104 : 62	$\chi^2_{1:2:1} = 3.81$	(df=2)	P=0.15
B1	148 : 61	$\chi^2_{3:1} = 5.86$	(df=1)	P=0.016

Linkage chi square test
$\chi^2_{3\times2} = 2.74$ (df=2) P=0.10

B1 – stands for the heterozygous and homozygous dominant genotypes

Table 4. Segregation for the genes *B1* and *Dw1* showing linkage

Cross F2 (R80×M57)
Genotype $\frac{b1,\ dw1}{B1,\ Dw1} \times \frac{b1,\ dw1}{B1,\ Dw1}$

Genotype and number of progeny		
	Dw1	*dwdw1*
B1–	121	46
b1b1	19	45

Segregation chi square tests				
Dw1	140 : 91	$\chi^2_{3:1} = 25.55$	(df=1)	$P < 10^{-3}$
B1	167 : 64	$\chi^2_{3:1} = 0.90$	(df=1)	P=0.34

Linkage chi square test
$\chi^2_{2\times2} = 35.45$ (df=1) $P < 10^{-3}$

Dw1 – and *B1* – stand for the homozygous dominant and heterozygous genotypes

between *An3* and *prxC*, and *Dw1* and *prxC* were found to be 23.5 ± 2.0 cM and 21.9 ± 1.6 cM, respectively, using the product method (Immer 1930; Mather 1938).

Another gene known to be located on chromosome IV is *B1*. Maizonnier and Moessner (1979) located *B1* on chromosome IV by trisomic segregation. From the analysis of the F2 (S1×R51) we found no reason to assume linkage of *prxC* to *B1* (Table 3). However, from the analysis of F2 (R80×M57) linkage of *B1* to *Dw1* was found. The genetic distance between *B1* and *Dw1* was found to be 26.9 ± 1.9 cM (Table 4).

We conclude that *prxC* is located on chromosome IV and the gene order is *B1-An3/Dw1-prxC*.

Location of prxC by Use of Trisomics

The available trisomic IV B3014 is heterozygous for *prxC*, containing the alleles *prxC1* and *prxC2*, one of the alleles in duplicate. The ratio of activity PRXc1/PRXc2 appeared to be dependent on the organ sampled and the developmental stage of organ and plant. In young leaves of young flowering plants the PRXc2 enzymes could barely be detected (due to overstaining of PRXc1) (Fig. 6), whereas in old tissue the allozymes have a similar activity. The temporal programming difference between the alleles *prxC1* and *prxC2* makes it impossible to conclude which is present in duplicate.

Table 5. Trisomic segregation for *prxC*

Cross	trisomic IV B3014×R74
Genotype	$\frac{prxC1}{\frac{prxC1}{prxC2}} \times \frac{prxC2}{prxC2}$
Genotypes and number of diploid progeny	
prxC1/C2	76
prxC2/C2	42
Segregation chi square tests (df=1)	
$\chi^2_{1:1}=9.8$	$P<10^{-3}$ $\chi^2_{2:1}=0.27$ $P=0.60$

To construct triply heterozygous trisomics, the trisomic IV was crossed to the line A4 (*prxC3/C3*). Among the selected trisomic progeny two PRXc phenotypes were found: PRXc1/c2/c3 (3 plants) and PRXc1/c3 (4 plants). We conclude that the genotype of plants with the latter phenotype is *prxC1/C1/C3*, and, consequently, that the trisomic IV contains two *prxC1* alleles and a *prxC2* allele.

To study trisomic segregation for prxC, the trisomic IV B3014 was also crossed to line R74 (*prxC2/C2*). The results shown in Table 5 indicate, again, the presence of three *prxC* alleles in the trisomic IV.

Differential Expression of prxC1 Alleles in Triply Heterozygous Trisomics IV

Among the progeny of the cross trisomic IV B3014×A4 two types of triply heterozygous trisomics IV could be distinguished by the different temporal expression of the *prxC1* alleles of the trisomic IV B3014 (*prxC1/C1/C2*) with regard to that of the *prxC2* allele. In one type (1 plant) the PRXc1 enzymes in mature corolla tissue of young flowering plants are less active than the PRXc2 enzymes, but in leaves are more active (Fig. 6a). For the other type (2 plants) the situation is reversed. In flower tissue a higher expression level of the *prxC1* allele was found, but in leaf tissue a lower level. During aging of tissue and plant the expression of all three alleles reached a similar level.

Differential Expression of prxC Alleles in the Progeny of Trisomic IV×R74

On the basis of PRXc phenotype of corolla tubes the plants of the progeny trisomic IV B3014×R74 (genotype *prxC1/C1/C2×prxC2/C2*) can be divided into three groups (Figs. 7 and 8):

1. plants with genotype *prxC2/C2* (42 plants)
2. plants with genotype *prxC1/C2* which show a higher expression level of the *prxC1* allele relative to the *prxC2* allele (40 plants)

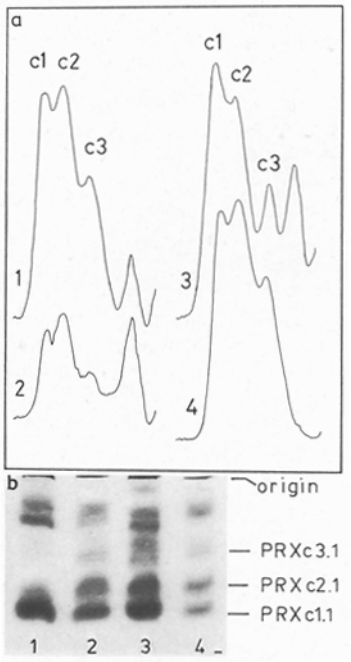

Fig. 6a and b. Densitometric scannings (**a**) and system II starch gel (**b**) showing the differential expression of the alleles *prxC1*, *prxC2*, and *prxC3* in triply heterozygous trisomics IV obtained from the cross trisomic IV B3014×A4, and in the trisomic IV B3014. **a** The origin of the scannings is at the right, and only the allele numbers are indicated. (1), corolla tube; (2), bud; (3), leaf; (4) limb. **b** (1), trisomic IV B3014, *prxC1/C1/C2*; (2), (3), (4) trisomics IV, *prxC1/C2/C3*; (2), (3), show a higher and (4) a lower expression level of the *prxC1* allele compared to *prxC2*. Samples were leaves of flowering plants

3. plants with genotype *prxC1/C2* which show a lower expression level of the *prxC1* allele relative to the *prxC2* allele (36 plants).

The division of the *prxC1/C2* heterozygotes in two groups was possible by measuring peak heights of densitometric tracings of PRXc bands obtained by zymographing mature corolla tubes taken from plants of comparable age (Fig. 8). We assumed that the tube presents a developmentally defined tissue, as could be checked by comparing the intensity of the anodic peroxidases. The PRXa and PRXb bands are more constant in activity than the PRXc bands (Fig. 7). The differential expression of the *prxC* alleles involved appeared to be highly dependent on the age of the plants: analysis of the progeny about a month later showed that the separation of the two *prxC1/C2* groups was less evident, whereas in mature corolla tubes of old plants the alleles *prxC1* and *prxC2* showed a similar expression level. The 1:1 segregation for the PRXc phenotypes may indicate the presence of three different *prxC* alleles in the trisomic B3014, but it cannot yet be concluded whether an internal or external site mutation is involved. In any

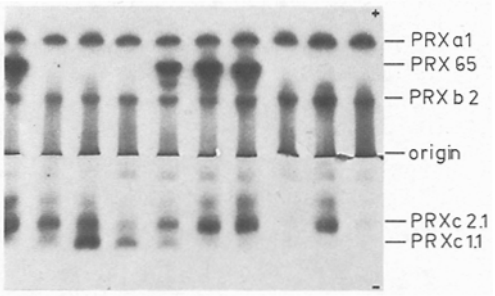

Fig. 7. Starch gel showing analysis of the progeny trisomic IV B3014×R74. Samples were corolla tubes taken at about two weeks after the onset of flowering. Only symbols for the faster moving bands of the PRXc doublet are given. PRX65 denotes a flower peroxidase according to the initial nomenclature (van den Berg and Wijsman 1981)

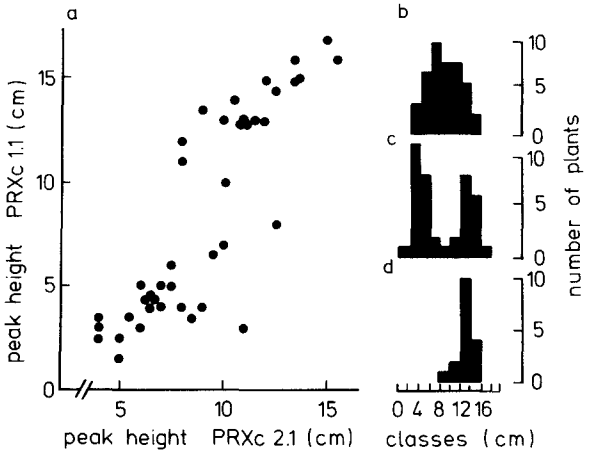

Fig. 8 a–d. Graphical representation of the zymographical analysis of corolla tubes of the progeny of trisomic IV B3014×R74. **a** plot of PRXc2.1 versus PRXc1.1 activity showing separation of the *prxC1/C2* heterozygotes into two groups. **b–d** histographical representation of PRXc activity (peak height, in classes of 2 cm) of the three groups: b. PRXc2.1 activity of *prxC1/C2* heterozygotes; c. PRXc1.1 activity of *prxC1/C2* heterozygotes; d. PRXc2.1 activity of *prxC2* homozygotes

case we can say that the expression of the *prxC* alleles in the progeny of B3014×R74 is determined by at least three different temporal programmes.

Differential Allelic Expression affects the Activity of Both PRXc Doublet Bands

From monogenic inheritance and concomitant shifting of the PRXc doublet bands, it was concluded that both bands of the doublets are encoded by one structural gene (van den Berg and Wijsman 1981). A third line of evidence is presented here by differential allelic expression. For example, when the enzyme PRXc2.1 shows a higher activity than the PRXc1.1 enzyme, the PRXc2.2 enzyme then also shows a higher activity than the PRXc1.2 enzyme (e.g., Fig. 3, lane 5).

For the differential expression of the *prxC2* and *prxC3* alleles the presence of an internal site mutation can be concluded to. Plants with genotype *prxC2/C3* from different progenies consisting of more than 300 plants consistently showed in young tissue a lower expression level of the *prxC3* allele. The internal site mutation affects the activity of both bands of the PRXc3 doublet compared to the activity of the PRXc2 doublet. This means that both doublet bands are under the control of the same cis-acting mutation, and, consequently, that both bands are coded for by one structural gene.

Discussion

The gene *prxC* could be located on chromosome IV by its linkage to the genes *Dw1* and *An3*, by trisomic segregation, and by the construction of triply heterozygous trisomics IV. We have one telotrisomic that possibly is a telotrisomic IV (a gift from Dr. Maizonnier), so that a start can be made in locating of the genes on chromosome IV in relation to the centromere and termini.

Three lines of evidence showed that the PRXc doublets are encoded by the gene *prxC*. Previously, we reported the shift of the doublets for the alleles *prxC1* and *prxC2*, and the monogenic inheritance of both bands of the doublets (van den Berg and Wijsman 1981). Now, we can add a third variant for which the doublet shift still holds; and the absence of recombinant non-doublet types can be extended to progeny consisting of more than 3,000 plants. The third line of evidence comes from differential expression of alleles. The evidence is strengthened by the fact that one of the examples of differential allelic expression, namely the lower expression level of the *prxC3* allele, is caused by a cis-acting internal site mutation and involves both bands of the doublet.

The production of two cathodic peroxidases by one allele was also found by Felder (1976) in barley. He also reported root specific variation for the two bands. It is interesting to consider the differences in resolution of the PRXc enzymes between the gel systems I, II and III. It is known that borate ions bind to glyco-compounds, and thus can alter the charge of glycoproteins. Characteristic for system II is a borate front moving from the cathode towards the anode. It may be that the increase in mobility of the PRXc enzymes and the doublet phenotype is caused by a difference in sugar content of the peroxidases.

In accordance with this is the fact that the increase of mobility can only be detected in very young tissue, i.e. flower buds. It must be noted that Fig. 4 shows two bud specific bands in buds of plants with genotype *prxC1/C1*. Clearly, more research is needed to elucidate the developmental expression of the gene *prxC*.

The difference in temporal expression of the two *prxC1* alleles of the trisomic IV B3014 was first recognized in material of the triply heterozygous trisomics IV. The analysis of the progeny of the cross trisomic IV × R74 showed mendelian segregation for the two *prxC1* alleles involved. It cannot yet be concluded whether the difference in temporal expression of the *prxC1* alleles is caused by an internal site or an external site mutation. Further testcrosses to clear this matter are underway. However, the reciprocal effect of the differential expression of the two *prxC1* alleles for leaf and flower tissue of the triply heterozygous trisomics IV, analogous to the situation of the *Adh-1* locus in maize (Woodman and Freeling 1981), may point to a regulatory mutation affecting the expression of the *prxC1* allele.

Further analysis of temporal programming differences of *prxC* alleles can be studied with the use of triply heterozygous trisomics. By appropriate crosses the temporal expression of a *prxC* allele can be compared to the expression (or expression ratio) of two other *prxC* alleles of the same plant.

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