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Genetics of the peroxidase isoenzymes in Petunia

11. Several loci involved in peroxidase synthesis

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Summary. The structural gene *prxE*, coding for a slow cathodic peroxidase in Petunia, has been located to chromosome II, linked to *Fl*. The presence of two mobility alleles in *Petunia hybrida* can be ascribed to its hybrid descent. Some properties of peroxidase *e* are mentioned. A gene *prxJ* is postulated for a still slower cathodic band. The gene *Rpl*, regulating the onset of expression of the allele *prxB2*, has been located on chromosome VII (gene order *Rpl-prxF-An4*). A synopsis of the isoperoxidases and the corresponding genes is given.

Key words: Petunia – Plant peroxidase regulation

Introduction

Structural genes for the more conspicuous ("major") Petunia peroxidases in shoots (*prxA, prxB,* and *prxC*) as well as in roots (*prxG, prxH*) have already been described (van den Berg and Wijsman 1981, 1982a, 1982b; van den Berg et al. 1983, 1984b; Wijsman 1983) as well as a postulated additional one encoding the flower specific peroxidase *f*. In addition, a few isoenzymes migrating less far from the origin can be distinguished; one anodic, mentioned as PRX-22 by van den Berg and Wijsman (1981) and encoded by the structural gene *prxD* (Hartings and Wijsman 1984); two others cathodic, presently called peroxidase *j* (nearest to the origin) and *e*.

Van den Berg et al. (1983) have described the gene *Rpl* as influencing the onset of the expression of *prxB2*, but this gene has not been located.

This report is a survey of structural and regulatory genes represented in the collection of pure lines of Petunia from the Institute of Genetics, University of Amsterdam.

Material and methods

Plant material

Inbred lines and relevant genotypes are:

R12	*prxE1E1, flfl*
Vu6	*prxE2E2, flfl*
V23	*prxE1E1, F1F1*
V35	*prxE1E1, flfl*
S9	*prxE2E2, flfl*
S12	*prxE2E2, flfl*
S2	*Rp1Rp1, prxF1F1, an4an4, prxB2B2*
S6	*rp1rp1, prxF2F2, An4An4, prxB3B3*

The first four lines are *P. hybrida;* S2 is *P. axillaris* ssp. *axillaris*; S6 and S9 are *P. integrifolia* ssp. *inflata;* S12 is *P. integrifolia* ssp. *integrifolia.*

Electrophoresis

Sample preparation and electrophoretic separation of the peroxidase isoenzymes were essentially carried out as described previously (van den Berg and Wijsman 1981). For peroxidase *a* gel system I was used. For peroxidase *b* (to screen for *Rpl*) or for peroxidase *f* either gel system I or system II were used. For the separation of peroxidases E1 and E2, lithium borate buffer was used, modified slightly from Gottlieb (1973) as follows: gel buffer stock was 0.051 M Tris +0.013 M citric acid (pH 7.9); electrode buffer 0.038 M LiOH +0.138 M boric acid (pH 8.3). The final gel buffer was composed by mixing two parts gel buffer stock with one part freshly made electrode buffer and diluting 2½ times with demineralized water. Staining was carried out according to van den Berg and Wijsman (1981), but to obtain a better staining of peroxidase *e* the concentration of sodium acetate buffer was increased from 0.05 M to 0.2 M.

Other genetic markers

Phenotypes of *Fl* and *An4* (as mentioned in Wijsman and van den Berg 1982) were determined according to Wiering (1974).

Results

Location of the gene prxE

Young plants have one minor cathodic band near the origin of the electrophoresis, peroxidase *e*. Older plants have an additional, faster band (a mozyme, see van den Berg and Wijsman 1981). PRXe could be demonstrated in most plant parts. Two pure lines, Vu6 and S12 (as well as all other *P. integrifolia* lines investigated) possess a faster PRXe allozyme pair, as could be best distinguished on citrate/lithium borate gels according to Gottlieb 1973 (Fig. 1). The PRXe2 bands develop later than the PRXe1 bands. The two doublets behave as alleles in the selfing of a heterozygote, where the three combinations segregated as 14 : 24 : 13. This implies that *prxE* is the structural gene for the enzyme.

In order to locate gene *prxE*, several genetic markers representing all seven linkage groups were tested for linkage: 86% linkage with the factor *Fl* on chromosome II could be demonstrated (Table 1).

P. integrifolia line S9 (*prxE2E2*) has one single band regardless of the age of the plant. F1 hybrids with *prxE1E1* lines show two bands, PRXe1.1 as well as the coinciding PRXe1.2 and PRXe2.1. One explanation is that a modifying, mozyme-forming enzyme is allozyme-specific and that interaction between the modifying enzyme and the particular PRXe2 does not take place in S9. Later generations will show whether the defect resides in the peroxidase or in its modifier.

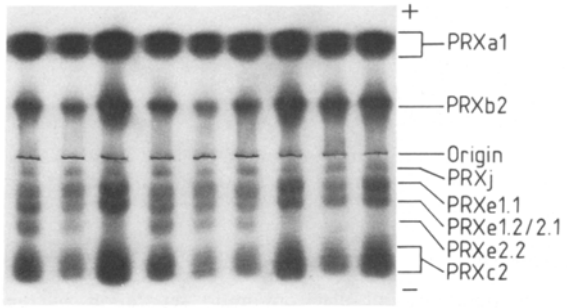

Fig. 1. Lithium borate starch gel showing segregation for *prxE1* and *prxE2* in the backcross (Vu6 × R12) × V35

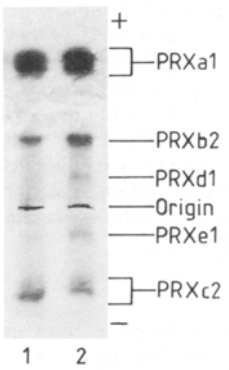

Fig. 2. System II starch gel showing the tissue specificity of PRXe in leaf tissue of *Petunia hybrida* (*prxE1E1*). *Lane 1:* leaf midrib; *lane 2:* leaf remaining tissue. Note the similar tissue specificity of PRXd

Table 1. Segregation of the genes *prxE* and *Fl* showing linkage

Cross: B1 (Vu6 × V23) × V35

Genotype B1: $\frac{prxE2, fl}{prxE1, Fl} \times \frac{prxE1, fl}{prxE1, fl}$

Phenotypes and number of progeny

	Flfl	*flfl*
prxE1E1	90	15
prxE1E2	11	72

Segregation chi square tests

prxE	105 : 83	$\chi^2_{1:1} = 2.57$	df = 1	P = 0.12
Fl	101 : 87	$\chi^2_{1:1} = 1.05$	df = 1	P = 0.31

linkage chi square test

$\chi^2_{2\times2} = 97.9$ (df = 1) $P \ll 0.001$ Distance 13.8 ± 1.7 cM

As to cellular localization, enzyme PRXe seems to be extracellular. When callus has been grown on agar, PRXe activity can be recovered from the agar after removal of the callus. The data of Hendriks et al. (1985) point to PRXe as an enzyme ionically bound to the cell wall which does not bind to concanavallin A. Extracts from leaf halfs were richer in activity than extracts from leaf midribs (Fig. 2).

Peroxidase J

In extracts of old plants a band can be found on a system II gel between the origin and PRXe (Rf: 0.05; Fig. 1). It could be demonstrated in all inbred lines and in all organs; in leaves the plant as well as the organ must be old for this PRXj band to be seen. Virus-infected leaves had a very high activity. This enzyme does not bind to concanavallin-A (Hendriks et al. 1985).

In heterozygotes for *prxA, B, C, D,* and *E,* the PRXj band remains single. In addition, it can be seen regardless of the presence of PRXf. As this enzyme can not be a mozyme of the isoenzymes already described, a separate gene *prxJ* must be postulated.

The trans-regulatory gene Rp1

Van den Berg et al. (1983) described a gene influencing the allozyme balance of the PRXb isoenzymes. In an Rp^+ background *prxB3* expression exceeds *prxB2* expression, which reflects the later start of the latter. In an rp^- background (*P. integrifolia* ssp. *inflata*), expression of *prxB2* starts earlier, and as a result both allozymes are more or less balanced in activity, often even showing a preponderance of *prxB2.* It was demon-

strated that *Rp* segregates in a monofactorial fashion, but the gene was not mapped.

In the F2 of lines S2×S6, in which each linkage group was represented by at least one marker gene, segregation for *Rp1* was observed in the F2, and linkage of *Rp1* to *prxF* (12 cM) could be demonstrated (Table 2). Unpublished data show *prxF* and *An4* to be more closely linked than was considered by van den Berg et al. (1984b), who may have come across an exceptional case. In the present cross, the distance of *prxF* and *An4* is 18±8 cM. *Rp1* does not segregate among $An4^+$ plants significantly different from the data in Table 2 (14:11 versus 24:19, respectively). It follows that the gene order to be deduced is *Rp1-prxF-An4*.

Discussion

The importance of the additional genetic data is that they complete the picture of the genetic control of the peroxidase isoenzymes in Petunia. We have tried to locate all structural genes. For *PRXf* and *PRXj* no structural variants are known. However, the gene *prxF* has been located by using a null allele, and may be a structural gene. That *prxD* is a structural gene (Hartings and Wijsman 1984) is based on circumstantial evidence only. The exceptional line W115 (*prxD2D2*, differing from all other lines investigated, all of genotype *prxD1D1*) could possibly have a null allele for *PrxD* in combination with a unique slower band. Only the fact that both PRXd1 and PRXd2 bands are H_2O_2 sensitive points to their allelic relationship.

As to the location of *prxE*, de Vlaming et al. (1984) published that some other genes were very closely linked to *F1* and only one gene was weakly linked (*Ws*, at 28 cM). Dietrich et al. (1981, Fig. 5) showed that one arm of chromosome II consists largely of heterochromatin. This points to a gene order *F1-prxE-Ws* because the alternative would endow one chromosome arm with nearly as many cM as the largest chromosomes IV and VII, with two euchromatic arms.

Power et al. (1976) showed the appearance of an additional band in the *PRXe* zone in sexual or somatic hybrids of *P. hybrida* and *P. parodii*. This additional band, absent from the zymogram of the parents, can now be interpreted as a hybrid vigour phenomenon, making the leaves more vigorous, that is, "older", and in this way inducing the PRXe mozyme earlier than in the inbred lines.

To further account for all the bands, we wish to mention three weak mozymes co-migrating with the prxB main bands, and not mentioned by van den Berg and Wijsman (1982a) because they are only visible in a system II gel (cf. van den Berg et al. 1982, Fig. 1). An additional enzyme of a very low Rf value but at the anodal side may be specific for the style (cf. van den Berg and Wijsman 1981).

According to Gottschalk (1953) the basal chromosome number in the Solanaceae is six. Species with 2n=24, like *Lycopersicon* or *Nicotiana*, might be re-diploidized tetraploids, while, by contrast, Petunia would have had only the addition of one chromosome (2n=14). There is no evidence for genome duplication in Petunia. We expect no duplicate forms of the peroxidases and there is evidence that all peroxidases are different at the peptide level. As for PRXa and PRXb it is known that their pI's are diametrically different, though the molecular weights are approximately the same (Hendriks, unpublished results). Immunological work on the homology of peroxidases in the Solanaceae is in progress.

Above, we have located the gene *Rp1* on chromosome VII. Clearly, it concerns an external site, active in

Table 2. Segregation for the genes *Rp1* and *prxF* showing linkage

Cross	F2 (S2×S6)			
Genotype F1:	$\frac{Rp^+, prxF1}{rp1, prxF2}$			
Phenotypes and number of progeny				
	prxF1–	*prxF2F2*		
Rp^+–	21	3		
rp1rp1	3	16		
Segregation chi square teste				
Rp	24:19	$\chi^2_{3:1}=8.44$	df=1	P<0.05
prxF	24:19	$\chi^2_{3:1}=8.44$	df=1	P<0.05
Linkage chi square test				
$\chi^2_{2\times2}=22.1$	(df=1)	$P \ll 0.001$	Distance 12.6±3.8 cM	

Rp^+– and *prxF1*– stand for the heterozygous and homozygous dominant genotype

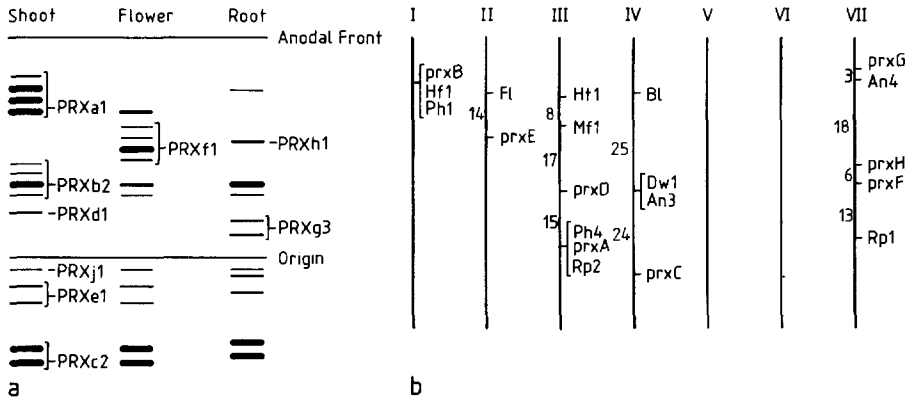

Fig. 3. a Schematic peroxidase patterns in shoots, flower, and root of Petunia. **b** Peroxidase gene linkage map; the figures are distances in cM. The chromosomes have been rendered as of equal length, since the positions of the genes relative to the centromere are unknown

trans, as *Rp1* regulates the onset of gene expression of *prxB* on chromosome I. Because it influences the *prxB2* allele but not the *prxB3* allele (van den Berg et al. 1983), *Rp1* is likely to have a specific effect on peroxidase development. In the same way another gene, presently called *Rp2*, is regulating *prxA6* but not *prxA1* (van den Berg et al. 1984a). Preliminary unpublished evidence, however, points to the gene *Rp2* being located on chromosome III, just as *prxA* itself.

The above leads us to a final interpretation of the peroxidase isozyme pattern in Petunia and an appraisal of the genetic situation, which are rendered in Fig. 3.

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