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Genetics of the peroxidase isoenzymes in *Petunia*

10. Location of the gene *prxD*

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Summary. The structural gene *prxD* in *Petunia* codes for a slow moving anodic peroxidase whose activity is sensitive to high concentrations of hydrogen peroxide. The PRXd enzyme could be found in mature and old leaf and stem tissue of full-grown flowering plants. PRXd was found to be absent in tissues from flower corolla and root. The gene *prxD* is the fourth gene that codes for peroxidases in leaf and stem. Two mobility variants of the PRXd enzyme have been found among our inbred lines using starch gel system II electrophoresis. The gene *prxD* could be located on chromosome III by a four-point-cross involving the genes *prxA, prxD, Mf1* and *Ht1*. The order of the genes established is: *Ht1* – *Mf1* – *prxD* – *prxA*.

Key words: *Petunia* – Peroxidase isoenzymes – Gene localization

Introduction

In previous papers we have described the chromosomal location of the genes *prxA, prxB* and *prxC* that code for the major peroxidases present in leaf and stem tissue (van den Berg and Wijsman 1982a, b; van den Berg et al. 1982; Wijsman 1983), the gene *prxF* encoding a peroxidase present only in the flower corolla, and the genes *prxG* and *prxH* that are expressed in roots only (van den Berg et al. 1984).

In our first paper of this series we mentioned a peroxidase from leaf tissue with a slightly lower mobility than PRXb. Since no genetical analysis was possible, we named the band PRX22 (van den Berg and Wijsman 1981). Recently we have found a slower moving variant of this PRX22 in a line derived from the doubled haploid *Petunia* Mitchell. This gave us the possibility to begin analysis on the chromosomal position of the peroxidase gene, now named *prxD,* that codes for the PRX22 enzyme and the slower moving variant.

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Materials and methods

Plant material

The following inbred lines were used:

- W115: *prxA1/A1, prxD2/D2, mf1 mf1, Ht1 Ht1*
- V23: *prxA2/A2, prxD1/D1, Mf1 Mf1, ht1 ht1*
- V35: *prxA1/A1, prxD1/D1, mf1 mf1, ht1 ht1*

Plant W115 is a doubled haploid derived from a cross involving *P. hybrida* cultivar 'Rose of Heaven' and a *P. axillaris* plant (Mitchell et al. 1980). Plants were grown in the greenhouse under standard flower inducing conditions.

Electrophoretic analysis

Sample preparation and electrophoretic separation of the peroxidase a isoenzymes using gel system I were carried out as described previously (van den Berg and Wijsman 1982a). Electrophoretic separation of the peroxidase d isoenzymes using gel system II was carried out essentially as described previously (van den Berg and Wijsman 1981). The electrophoretic procedure was modified in changing the electrode buffer normally containing 0.3 M boric acid into 0.5 M boric acid. Peroxidase staining was carried out essentially as described in van den Berg and Wijsman (1981). A modification consisted in adding only 25 µl 30% H_2O_2 instead of 100 µl 30% H_2O_2 to the staining solution in order to obtain a better PRXd staining.

Determination of flower colour gene segregation

Determination of genotypes for *Mf1,* a methylation gene controlling substitution at the 3′ and 5′ end positions of anthocyanins, and *Ht1,* a hydroxylation gene controlling substitution of a hydroxyl group at the 3′ position of anthocyanins, was carried out as described by Wiering (1974).

Nomenclature

The nomenclature as described by van den Berg and Wijsman (1981) is followed here: the structural gene encoding both peroxidase d variants is termed *prxD;* alleles and enzymes are numbered, e.g. the allele *prxD1* encodes the isoenzyme PRXd1.

Results and discussion

Variants and properties of the peroxidase d enzymes

The peroxidase d enzymes were found in mature and old leaf tissue of mature plants. PRXd was not detected in flower corolla and root tissue. Peroxidase d enzymes can be characterized by two properties. First, of the three gel systems normally used by us, only a system II gel consistently shows PRXd bands (see zymograms in: van den Berg and Wijsman 1981, 1982a, b). Occasionally PRXd bands can also be seen on system I gels. We do not know the cause of this phenomenon. Clearly PRXd cannot be considered to be hidden under PRXb since variation of the mobility of PRXb does not give rise to the presumed hidden PRXd (see Fig. 3 in van den Berg et al. 1983). Resolution of the PRXd could be improved by modification of gel system II (cf. Fig. 4b in van den Berg and Wijsman 1981 and Fig. 1 this paper). Secondly, the activity of the PRXd enzyme depends greatly on the concentration of hydrogen peroxide used during staining. The concentration normally used, which has no detectable influence on the activity of the peroxidases a, b and c, inhibits the activity of the peroxidase d enzyme. The inhibition, however, is not the same for both PRXd isoenzymes. It has been found that the isoenzyme PRXd2 is much more sensitive to high concentrations of hydrogen peroxide than the isoenzyme PRXd1. Staining at too high concentrations of hydrogen peroxide therefore can lead to wrong conclusions drawn from experiments since *prxD1/D2* heterozygotes appear like *prxD1/D1* homozygotes, due to selective inhibition of the PRXd2 enzyme.

Table 1. Four point cross involving the genes *prxD, prxA, Ht1* and *Mf1*, showing linkage

Cross: B1 (W115 × V23) × V35

Genotype: $\frac{Ht1\ Mf1\ prxD1\ prxA2}{Ht1\ mf1\ prxD2\ prxA1} \times \frac{ht1\ mf1\ prxD1\ prxA1}{ht1\ mf1\ prxD1\ prxA1}$

Genotypes and numbers of progeny found

	prxA1/A1 prxD1/D1	*prxA1/A2 prxD1/D1*	*prxA1/A1 prxD1/D2*	*prxA1/A2 prxD1/D2*
Ht1 ht1 Mf1 mf1	0	4	0	0
Ht1 ht1 mf1 mf1	3	8	40	7
ht1 ht1 Mf1 mf1	6	37	9	6
ht1 ht1 mf1 mf1	0	0	3	1

Segregation chi square tests

prxD	66:58	$\chi^2_{1:1}=0.52$	$P=0.47$
prxA	63:61	$\chi^2_{1:1}=0.03$	$P=0.82$
Ht1	62:62	$\chi^2_{1:1}=0$	$P=1$
Mf1	62:62	$\chi^2_{1:1}=0$	$P=1$

Linkage chi square tests

prxA–prxD	14:52:49: 9	$\chi^2_{1:1:1:1}=49.6$	$P<10^{-3}$
prxA–Mf1	14:47:47:16	$\chi^2_{1:1:1:1}=33.1$	$P<10^{-3}$
prxA–Ht1	18:43:44:19	$\chi^2_{1:1:1:1}=20.2$	$P<10^{-3}$
prxD–Mf1	11:47:52:14	$\chi^2_{1:1:1:1}=44.7$	$P<10^{-3}$
prxD–Ht1	15:43:47:19	$\chi^2_{1:1:1:1}=25.8$	$P<10^{-3}$
Ht1–Mf1	4:58:58: 4	$\chi^2_{1:1:1:1}=94.1$	$P<10^{-3}$

Genetic distances

prxA–prxD	18.5 ± 3.4 cM
prxD–Mf1	21.0 ± 3.6 cM
Mf1–Ht1	6.5 ± 2.2 cM
prxA–Mf1	25.0 ± 3.9 cM
prxD–Ht1	27.4 ± 4.0 cM
prxA–Ht1	29.8 ± 4.1 cM

All inbred lines as well as plants of the species *P. axillaris* and *P. integrifolia* from the *Petunia* collection of the Institute of Genetics, Amsterdam, investigated, are homozygous for the allele *prxD1*. Recently, however, a slower moving variant, PRXd2, has been found in plants of *Petunia* Mitchell (Mitchell et al. 1980). These plants were named W115 and are homozygous *prxD2/D2*.

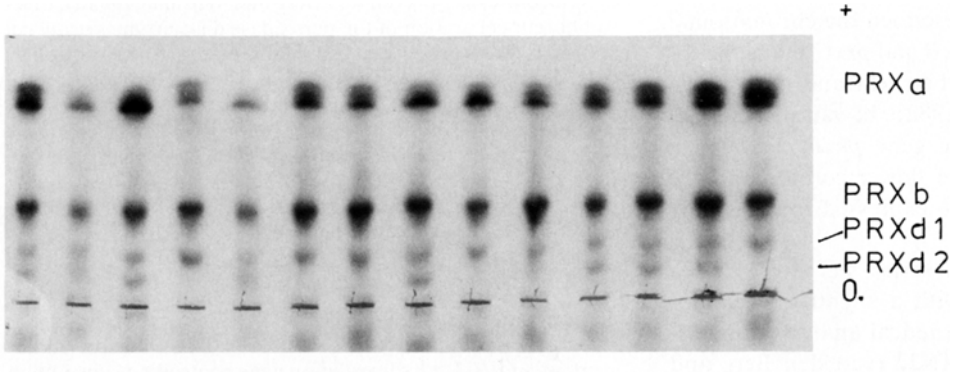

Fig. 1. System II starch gel showing segregation of the gene *prxD* among the progeny of the backcross (W115 × V23) × V35. Old leaves of mature plants were analysed. Segregation found *prxD1/D1:prxD1/D2* = 58:66 ($\chi^2_{1:1}=0.52$, $P=0.47$)

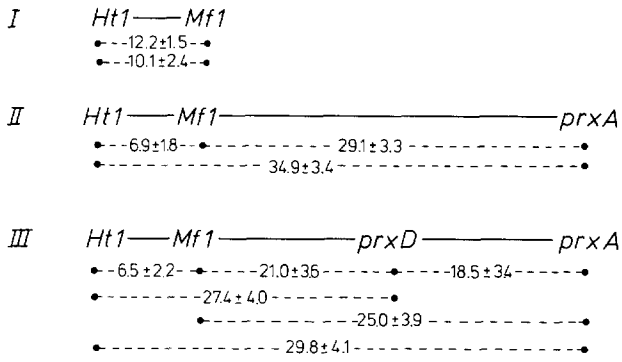

Fig. 2. Genetic map of the genes on chromosome III. The data are derived from Wiering and de Vlaming (1977) (*I*), van den Berg and Wijsman (1982b) (*II*) and Table 1 (*III*)

Location of the gene prxD

Analysis of the backcross (W115×V23)×V35 showed linkage of the gene *prxD* to the genes *prxA, Mf1* and *Ht1*. The genes *Ht1* and *Mf1* have been shown by Wiering and de Vlaming (1977) to be linked and to be located on chromosome III (Maizonnier and Moessner 1979). Van den Berg and Wijsman located the gene *prxA* on chromosome III, showing linkage between the gene *prxA* and the genes *Ht1* and *Mf1* (van den Berg and Wijsman 1982b). We therefore conclude that the gene *prxD* is located on chromosome III. We assume that the order of the genes on chromosome III is: *prxA – prxD – Mf1 – Ht1*, based on the data given in Table 1. This assumed gene order gives in addition to single and double crossovers no triple crossovers, whereas other gene orders would assume triple crossovers to exist as well as higher numbers of single and double crossover. From Fig. 2 can be seen that the genetic distances found between the genes *prxA, Mf1* and *Ht1* are in good agreement with the data found earlier by van den Berg and Wijsman (1982b).

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