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Genetics of the peroxidase isoenzymes in *Petunia*

7. The alleles *prxA6* and *prxA7*

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Summary. Two alleles of the structural gene *prxA* from *Petunia, prxA6* and *prxA7,* could be identified by their differential temporal expression. The alleles *prxA6* and *prxA7* code for peroxidases with a similar electrophoretic mobility as the products of the previously described alleles *prxA1* and *prxA5,* respectively. The former two alleles differ in that they have a different temporal expression with regard to the temporal expression of the allele *prxA2.* Crossing experiments indicated that the mutations involved are (cis-acting) internal site mutations. In the case of the allele *prxA6,* the experiments indicated a difference with respect to the allele *prxA1* in responsiveness to the action of a trans-acting factor.

Key words: *Petunia* – Peroxidase – Differential temporal expression

Introduction

The five alleles, *prxA1* to *prxA5* of the structural gene *prxA* in *Petunia* could be identified by the electrophoretic mobility of the encoded enzymes. PRXa allozyme balance analysis of plants from F1, B1, and F2 progenies segregating for *prxA* mobility alleles resulted in the description of the five different temporal programmes that are characteristic for each of the five mobility alleles. These crossing experiments showed that internal site mutations are involved in the differences in temporal programming of the five mobility alleles.

In addition to mutants that share a mobility mutation and temporal programming mutation (which may be identical, though unlikely) we investigated the potential presence of internal site mutations among alleles that code for peroxidases with similar mobility. This was possible by taking the temporal expression of another mobility allele as a reference. This approach resulted in the identification of two other *prxA* alleles that could only be identified by their temporal expression. These two alleles, *prxA6* and *prxA7,* code for peroxidases that are probably identical to the peroxidases encoded by the alleles *prxA1* and *prxA5,* respectively.

Materials and methods

The plants used were: *P. hybrida* lines V23, W4 and R51; *P. axillaris* ssp. *axillaris* line S2; *P. integrifolia* ssp. *inflata* line S6 and plant D580-2; and *P. integrifolia* ssp. *integrifolia* plant D660-1. The origin of these plants and their taxonomic allocation has been given earlier (van den Berg and Wijsman 1982a, b; Wijsman 1982).

The *prxA5* allele of D660-1 (*prxA2/A5*) can be found in line S12; the *prxA7* allele of D580-2 (*prxA2/A7*) can be found in line S17.

Sample preparation, electrophoretic separation of the peroxidase isoenzymes, and staining for peroxidase activity were carried out as described previously (van den Berg and Wijsman 1982b).

The same nomenclature as used in previous papers has been followed (van den Berg and Wijsman 1982a, b). For instance, the allele *prxA1* encodes the PRXa1 enzyme variant. The allele *prxA6* encodes what is probably the same enzyme, but the *prxA6* allele shows a higher expression level in young tissue, caused by an internal site mutation. For the sake of clarity the enzymes encoded by the allele *prxA6* all receive the same number, though they may be identical to the PRXa1 enzymes.

Results and discussion

The allele prxA6

P. hybrida line W4 apparently has genotype *prxA1/A1* as indicated by starch gel electrophoresis. However,

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genetic experiments led to the conclusion that the *prxA* allele from W4 shows a difference in temporal expression with regard to the standard *prxA1* allele from line R51. Since mutation of the gene *prxA* is involved, the allele from W4 was named *prxA6*.

Analysis of the F1 (W4 × S6) with genotype *prxA2/A6* showed that the *prxA6* allele had a delayed expression with regard to that of the *prxA2* allele, and similar to the delayed expression of the *prxA1* allele in the F1 (R51 × S6) with genotype *prxA1/A2:* in young leaf tissue of young plants both the PRXa6.1 and the PRXa1.1 enzyme have a much lower activity than the PRXa2.1 enzyme (see Fig. 2 in van den Berg et al. 1983a). However, in the F1 (W4 × S6), the PRXa6.1 enzyme showed a faster increase in activity relative to the PRXa2.1 activity, when compared to the developmental increase of the PRXa1.1 activity in the F1 (R51 × S6). The variant temporal programme of the *prxA6* allele from W4 could easily be scored by analysis of young leaf tissue of young plants at the onset of flowering. For *prxA6* expression it holds that

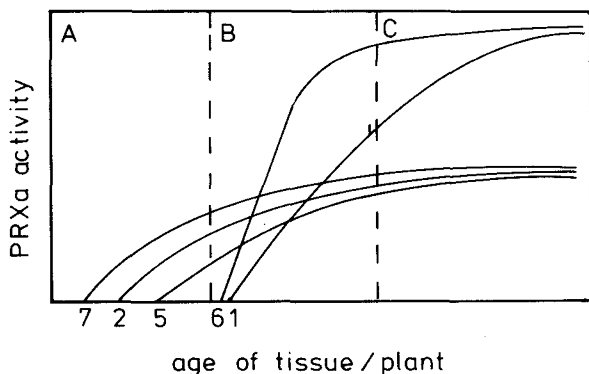

Fig. 1. Diagrammatic presentation of the differential expression of *prxA* alleles (only the allele numbers are indicated). The diagram is not based on the measurement of enzyme activity, but only on allozyme balance studies. It serves to illustrate the differences in start of expression and differences in increase of PRXa activity in tissue of different ages from young and older plants. *Zone A:* young leaves of young plants; *Zone B:* young leaves of mature flowering plants and mature leaves of young plants; *Zone C:* mature and older leaves of mature flowering plants

the final maximal difference between the activity of the PRXa6.1 and PRXa2.1 enzymes was already reached before flowering of the plant. By contrast, in the case of the F1 (R51 × S6), the final maximal difference in activity of the PRXa allozymes was only found in old leaves of old flowering plants (Fig. 1).

Though there is a clear difference in temporal expression between the alleles *prxA6* and *prxA1,* there is no or little difference in initiation time of *prxA* expression. In old tissue the difference between the expression level of the *prxA6* allele and the *prxA1* allele appeared to be similar to the difference between the expression level of the alleles *prxA1* and *prxA2* (a two- to three-fold higher activity of PRXa1.1).

Among the progeny of the backcross (W4 × S6) × S6 all *prxA3/A6* heterozygotes (42 plants) showed the variant temporal programme found in the F1 parent (the alleles *prxA2* and *prxA3* have a comparable expression level in mature leaf tissue, Fig. 2). However, all *prxA6/A2* heterozygotes (12 plants) among the progeny of the backcross (W4 × S6) × W4 showed the standard *prxA1* temporal programme. This indicated that line S6 contains a dominant trans-acting factor for which W4 is recessive, and that the variant *prxA6* temporal programme can only be expressed when the trans-acting factor is present in the dominant state.

In the progeny of the cross (W4 × S6) × W4, segregation for the trans-acting factor was to be expected. Since among the *prxA* heterozygotes of the progeny no segregation was found for the trans-acting factor, this could point to a linkage of the recessive allele of the trans-acting factor to the *prxA6* allele in W4. Preliminary experiments with a *prxA3/A6* plant from the progeny of the cross (W4 × S6) × S6 show Mendelian segregation for the variant and standard temporal programme, indicating heterozygosity of the *prxA3/A6* parent for the trans-acting factor. The possibility of linkage of the factor to *prxA* is currently under investigation.

Since the F1 (R51 × S6) did not show the variant temporal programme (and also no *prxA1/A2* plants from the F2 progeny), we assume that the *prxA1* allele from R51 differs from the *prxA6* allele from W4 in that it is not responsive to the action of the trans-acting factor, and that an internal site mutation is involved.

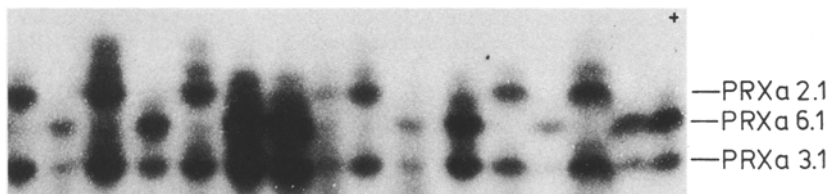

Fig. 2. Starch gel showing differential expression of the alleles *prxA2, prxA3,* and *prxA6* in mature leaf tissue of young plants from the progeny of the cross (W4 × S6) × S6. Note the comparable expression level of the alleles *prxA2 and prxA3,* and the higher activity of PRXa1.1 relative to PRXa3.1. Only the primary bands, not the modification products, of the three PRXa complex variants are indicated

The allele prxA7

The allele *prxA7* was found in plant D580-2 (*P. integrifolia* ssp. *inflata*). The experiments described below show that the *prxA7* allele encodes PRXa enzymes with similar electrophoretic mobility as those encoded by the allele *prxA5*, but with a different temporal expression.

Initially, genotype *prxA2/A5* for plant D580-2 was assumed as indicated by starch gel electrophoresis. Differential expression of the two alleles from D580-2 seemed to be similar to that of *prxA2/A5* heterozygotes that have the *prxA2* allele from line V23 and the *prxA5* allele from plant D660-1 (*P. integrifolia* s.s.) (a higher initial expression level of the allele *prxA2*, Fig. 1). From these observation there was no reason to assume any difference between the *prxA7* allele from D580-2 and the *prxA5* allele from D660-1. Moreover, both the *prxA7* and the *prxA5* allele showed a similar earlier start in expression with regard to the *prxA1* allele. This could be demonstrated by allozyme balance analysis of *prxA* heterozygous plants from the progeny of the crosses D580-2 × S2 (genotype *prxA2/A7*×*prxA1/A1*) and trisomic III × D660-1 (genotype *prxA1/A2/A3*× *prxA2/A5*).

From these experiments it would be logical to conclude that plant D580-2 contained alleles identical to the allele *prxA2* from line V23 and the *prxA5* allele from D660-1. However, among the progeny of V23 × D580-2 (*prxA2/A2*×*prxA2/A7*), all *prxA2/A7* plants showed a higher expression level in young leaf tissue of the *prxA7* allele with regard to the *prxA2* allele, in contrast to *prxA5* from D660-1 (Fig. 1, 3 b). Two *prxA2/A7* plants from this progeny were crossed and among the resulting plants all *prxA2/A7* plants (21 plants) showed the same specific differential temporal programming as the parents.

From these experiments we conclude that the *prxA7* allele from D580-2 is different from the *prxA5* allele from D660-1, and that it contains an internal site mutation that is responsible for a higher initial expression level of the *prxA7* allele with regard to the *prxA2* allele from V23 (Fig. 1).

It might be that the aberrant behaviour of the *prxA7* allele is caused by a trans-acting mutation present in D580-2. In that case, however, segregation for the *prxA5* allele having a lower or higher expression level with regard to *prxA2* would have been expected among heterozygotes from the progeny of the cross (D580-2 × V23) × (D580-2 × V23) (in that case with genotype *prxA2/A5*×*prxA2/A5*). It also would be possible that an external site mutation was present in plant D660-1 causing the lower expression level of the *prxA5* allele with regard to the *prxA2* allele. However, in further crossing experiments with *prxA2/A5* heterozygotes from the progeny of the cross trisomic III × D660-1, all *prxA2/A5* heterozygotes showed a higher expression level of the *prxA2* allele in young leaf tissue. Thus, the presence of an internal site mutation in D580-2 that differs from the one in D660-1 is more likely than the presence of an external site mutation. Two new lines, S12 (*prxA5/A5*) and S17 (*prxA7/A7*) were constructed from plants D580-2 and D660-1.

We have now the following order of increasing expression level of *prxA* alleles as found in young leaf tissue from young plants: *prxA1* (R51, S2), *prxA6* (W4), *prxA4* (S15), *prxA5* (S12), *prxA2* (V23, S6), *prxA3* (S9), *prxA7* (S17) (Fig. 1; Fig. 9 in van den Berg et al. 1983 a).

As mentioned in the beginning of the present section, the *prxA2* allele from D580-2 has a higher

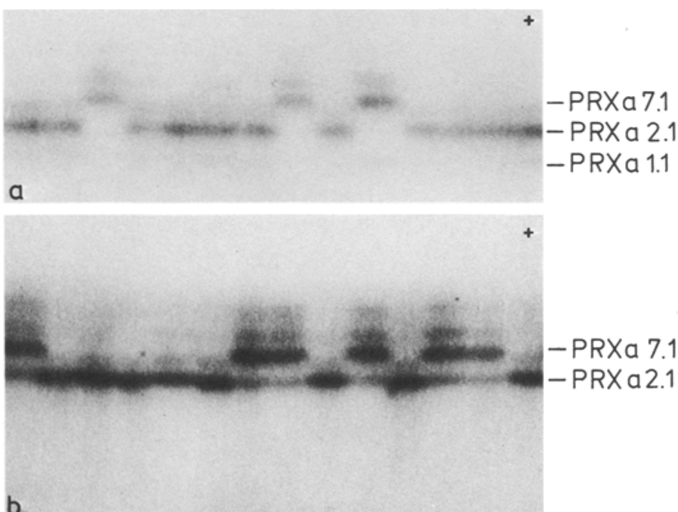

Fig. 3 a, b. Starch gels showing differential expression of the alleles *prxA1*, *prxA2*, and *prxA7*. **a** Analysis of young leaf tissue of young plants from the progeny of D580-2 × S2 (genotype *prxA2/A7*×*prxA1/A1*). All plants are heterozygous for *prxA*, *prxA1/A2* or *prxA1/A7*, but the *prxA1* allele was barely expressed in the young leaf tissue. **b** Analysis of mature leaf tissue of young plants from the progeny of D580-2 × V23 (genotype *prxA2/A7* ×*prxA2/A2*). Note the higher expression level of the *prxA7* allele (cf. Fig. 7 in van den Berg et al. (1983 a) which shows a lower expression level of the *prxA5* allele from D660-1 with regard to that of the *prxA2* allele from V23). Only the primary bands of the PRXa variants are indicated

initial expression level than the *prxA7* allele (in contrast to the *prxA2* allele from V23). Preliminary experiments suggest that D580-2 and V23 share identical *prxA2* alleles, but that an external site mutation present in D580-2 caused the higher expression level of the *prxA2* allele.

The examples, as shown above and also described in a former paper (van den Berg et al. 1983 b), of alleles encoding enzymes with similar electrophoretic mobility but with a different temporal programme may indicate that the *prxA* locus is made up of a structural element with adjacent regulatory regions. Mutation within the structural element can cause a change in mobility of the encoding peroxidase, whereas mutation in regulatory regions may cause change of the temporal programming. A similar situation was described by Chovnick et al. (1976) for the organisation of the *rosy* locus in *Drosophila melanogaster,* and, in the case of *Petunia,* for the *An1* locus, which is involved in the anthocyanin synthesis in the flower (Bianchi et al. 1978; Doodeman et al. 1984).

The temporal programming differences described here as well as in former papers dealing with differential expression of *prxA* alleles (van den Berg and Wijsman 1982 b; van den Berg et al. 1983 b) reflect differences in enzyme quantities in different stages of development of the plant. To investigate whether in addition to differences in enzyme quantities differences in specific peroxidase activity are also involved, the PRXa1 enzymes have been purified (van den Berg and van Huystee, in preparation), and antibodies against the purified PRXa have been obtained (van den Berg et al. 1984). Quantitative measurements of PRXa protein using immunological techniques ar in progress.

References

Berg BM van den, Wijsman HJW (1982 a) Genetics of the peroxidase isoenzymes in *Petunia.* 2. Location and developmental expression of the structural gene *prxB.* Theor Appl Genet 61:297–303

Berg BM van den, Wijsman HJW (1982 b) Genetics of the peroxidase isoenzymes in *Petunia.* 3. Location and developmental expression of the structural gene *prxA.* Theor Appl Genet 63:33–38

Berg BM van den, Bianchi, F, Wijsman HJW (1983 a) Genetics of the peroxidase isoenzymes in *Petunia.* 5. Differential temporal expression of *prxA* alleles. Theor Appl Genet 65:1–8

Berg BM van den, Wijsman HJW, Bianchi F (1983 b) Genetics of the peroxidase isoenzymes in *Petunia.* 6. Differential temporal expression of *prxB* alleles. Theor Appl Genet 66:173–178

Berg BM van den, Chibbar RN, Huystee RB van (1984) A comparative study of a cationic peroxidase from peanut and an anionic peroxidase from petunia. Plant Cell Rep (in press)

Bianchi F, Cornelissen PTJ, Gerats AGM, Hogervorst JMW (1978) Regulation of gene action in *Petunia hybrida:* unstable alleles of a gene for flower colour. Theor Appl Genet 53:157–167

Chovnick A, Gelbart W, McCarron M, Osmond B, Candido EPM, Baillie DL (1976) Organization of the *Rosy* locus in *Drosophila melanogaster:* evidence for a control element adjacent to the xanthine dehydrogenase structural element. Genetics 84:233–255

Doodeman M, Boersma EA, Koomen W, Bianchi F (1984) Genetical analysis of instability in *Petunia hybrida.* 1. A highly unstable mutation induced by a transposable element inserted at the *An1* locus for flower colour. Theor Appl Genet 67:345–355

Wijsman HJW (1982) On the interrelationships of certain species of *Petunia.* 1. Taxonomic notes on the parental species of *Petunia hybrida.* Acta Bot Neerl 31:477–490