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Inheritance of leaf peroxidase isoenzymes in *Nicotiana alata* and linkage with the S-incompatibility locus

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Summary. Genetic analysis of peroxidase isoenzymes observed by electrophoresis shows that each of the two cathodic bands are controlled by one gene, respectively, P_I and P_{II}. Each gene has two allele forms; presence of activity (dominant) and absence of activity (recessive). The same situation is found for one anodic band; the three other anodic bands are controlled by a single gene with three active allele forms. No progenies seem to be produced from gametes $P_I^- P_{II}^-$ (no activity of P_I or P_{II}). Investigation of the incompatibility system and the isoperoxidases demonstrates that the loci P_I, P_{II} and S are located in the same chromosome. P_I is closely linked to the S locus (3 cM); the distance between P_{II} and the S locus is 34 cM.

Key words: *Nicotiana alata* – Peroxidase isoenzymes – Genetic analysis – Linkage

Introduction

Pandey (1967) analysed the style peroxidase in seven different S genotypes of *N. alata.* However, the number of analysed plants per genotype was not large enough (1 to 4) to allow conclusion of a unique peroxidase pattern for each genotype. It is possible that the relation between the S-genotypes and the peroxidase isozyme patterns found by Pandey (1967) was a non-specific one. These peroxidases may be coded for by closely linked genes instead of by the S-locus itself. Such S-linked genes have not been identified until now. Neither has the appropriate genetic analysis of these peroxidase been performed. Bredemeijer and Blaas (1980) demonstrated that the peroxidase (and esterase) isozyme patterns of *N. alata* styles and pollen extracts were independent of the S-genotypes. The latter authors (1981) revealed S-specific proteins in styles of *N. alata* which seemed to be different from the peroxidase enzymes. Attempts to identify S-genotypes by comparing total peroxidase activity in leaves of *Trifolium pratense* (Anderson et al. 1974) and peroxidase isozymes in leaves of *Lycopersicum peruvianum* (Bredemeijer 1977) were unsuccessful.

The present paper deals with the genetic analysis of leaf peroxidases in *N. alata.* This study clarifies the genetic linkage between the S-locus and two other loci that control the cathodic leaf peroxidases. It also demonstrates the independence of the S-locus from two other genes which code for anodic peroxidase isozymes.

Materials and methods

Plant material

Several families of *N. alata* (L.) Link and Otto, with well identified monogenic gametophytic S-incompatibility alleles, were used. Certain genotypes used as testers were cloned by vegetative propagation (cuttings). One line of self-compatible *N. langsdorffii* was also studied.

The alleles S_1, S_3, S_{F10} and S_{F11} came from Pandey's testers. S_a, S_e and S_x are functional alleles identified by us in our own collection and differ from S_1, S_3, S_{F10} and S_{F11}. $S'_a(s'_y)$ is a spontaneous pollen-part mutant from $S_a(S_y)$ which allows selfing in plants like $S'_a S_n$; S'_a pollen is not recognized but S_a pollen is not accepted by this genotype.

The experiments were performed upon the cloned genotypes described in Table 1; their peroxidase phenotypes are depicted in Fig. 1. Electrophoresis was systematically performed on seedling leaves at the same stage of development, grown in the same conditions in greenhouses of the Phytotron at Gif-sur-Yvette.

Electrophoresis technique for isoperoxidases

Leaves were washed and then ground by mortar and pestle in a volume (in ml) of classical extraction buffer (Tris HCl

Table 1. Origin and description of genotypes studied

Species	Plant no.	S-incompatibility alleles	Incompatibility	Origin
N. alata	8.6	$S'_aS'_y$	SC	Kew (UK)
	16.1	S_cS_e [a]	SI	Brookhaven (USA)
	16.2	S_aS_a [a]	SI	Brookhaven (USA)
	16.6	S_aS_a [a]	SI	Brookhaven (USA)
	67.5	$S_{F10}S_{F11}$ [b]	SI	New Zealand
	67.6	S_{F10} [b] S_x [a]	SI	New Zealand
	67.8	$S_{F10}S_{F11}$ [b]	SI	New Zealand
	68.6	S_1S_3 [b]	SI	New Zealand
	68.7	S_1S_1 [b]	SI	New Zealand
	68.8	S_1S_3 [b]		New Zealand
N. langsdorffii	3.7	S_cS_c	SC	Bergerac (France)

[a] Alleles determined by the Laboratoire de Génétique et Physiologie du Développement des Plantes, CNRS, 91190 Gif-sur-Yvette (France)
[b] Allele identified by K. K. Pandey (New Zealand) who kindly furnished the seeds
SC: self-compatible
SI: self-incompatible

0.05 M, pH 7.2, Polyclon AT, Mercaptoethanol, EDTA 1 mM) equal to their weight in grams. The ground leaves were maintained at 0 °C for 3 h and then centrifuged at 30,000 g for 10 min at 0.2 °C.

The starch gel (12%, Tris citrate buffer 0.05 M, pH 7.0) was heated, gas was removed using a vacuum pump, the gel was then poured into a mould at room temperature and one hour later placed in a coldroom.

For each sample 65 µl of supernatant was deposited on the surface of the gel. Electrophoresis took place vertically for 16 h at 130–140 V. The reservoir buffer was of the same composition as the extraction buffer. After electrophoresis separation, the gel was cut into two superimposable slabs. Revelation of isoperoxidases, according to the method of Shaw and Prasad (1970), was performed on the lower slab. A solution of 50% glycerol was used to fix the electrophoregram.

This technique revealed two cathodic bands noted I and II and four anodic bands noted 1 to 4. In our notation, (+) signifies presence of enzyme activity and (−) absence of activity.

Tests for genetic linkage between S-locus and the peroxidases

We used some appropriate crosses in which only one type of pollen gamete was compatible on the female S-genotype. Let us suppose we are studying a segregation at a locus P (P^+ dominant over P^-) in a $♀ \frac{S_1 P^-}{S_1 P^-} \times ♂ \frac{S_J P^+}{S_1 P^-}$ cross, where only the S_J pollen is compatible. If the S_J and P^+ alleles are located closely on the same chromosome, the segregation pattern of P^+ and P^- alleles in the progeny will reveal such a linkage. The proportion of P^-P^- genotype in the progeny will measure the recombination value (r) between S and P loci in absence of other genetic disturbances, as illustrated by this figure of gamete mating.

♀ \ ♂	$S_J P^+$	$S_J P^-$
$S_1 P^-$	$[P^+]$	$[P^-]$
	$1-r$	r

This type of test does not need a complete classical analysis of S-genotypes if a zero level of pseudo-compatibility is assumed. In other cases complete analysis of S-alleles, P genotypes and linkage between them was performed.

Results

Inheritance of anodic peroxidases (Table 2)

a) Isozyme 1. The data observed (Table 2A) agree with a one locus hypothesis in which the presence of this band (1^+) is dominant over its absence (1^-). As the segregation ratios fit well with (1:1) and (3:1), there is no

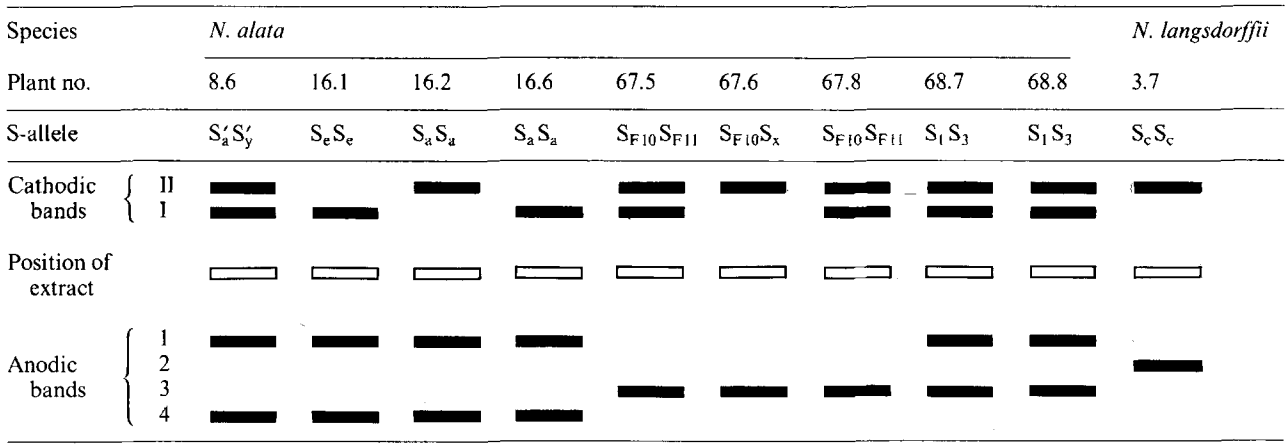

Fig. 1. Zymograms of plants studied

Table 2. Crosses and segregations observed for analysis of the inheritance of anodic isoperoxidases

A Segregation for $1^+/1^-$

Crosses	Genotypes suggested	Segregations observed for anodic peroxidase phenotypes		Theoretical segregation	χ^2 value
		$[1^+]$	$[1^-]$		
67.5 × (67.5 × 16.6)	$\left(\frac{1^-}{1^-}\right) \times \left(\frac{1^+}{1^-}\right)$	42	58	1:1	2.56 NS
8.6 × 94[a]	$\left(\frac{1^+}{1^-}\right) \times \left(\frac{1^-}{1^-}\right)$	21	29	1:1	1.28 NS
(68.8 × 67.5) selfed[b]	$\left(\frac{1^+}{1^-}\right)$	12	3	3:1	–
8.6 selfed	$\left(\frac{1^+}{1^-}\right)$	11	3	3:1	–

B Segregation for the 2, 3, 4 isozymes series

Crosses		Genotypes suggested	Segregations observed for anodic peroxidase phenotypes						Theoretical segregation	χ^2 value
			$[2^+3^-4^+]$	$[2^+3^+4^-]$	$[2^-3^+4^+]$	$[2^+3^-4^-]$	$[2^-3^-4^+]$	$[2^-3^+4^-]$		
Selfing of a hybrid $[2^+3^-4^+]$	(16.1 × 3.7)	$\left(\frac{2^+}{4^+}\right)$	74			40	27		2:1:1	
	(16.6 × 3.7)		23			17	20		2:1:1	
	(3.7 × 16.6)		31			20	9		2:1:1	
		Total	128			77	56		2:1:1	3.48 NS
Selfing[b] of a hybrid $[2^-3^+4^+]$	(67.5 × 16.6)	$\left(\frac{3^+}{4^+}\right)$			50		22	23	2:1:1	0.28 NS
Cross $[2^-3^+4^+]$ × $[2^+3^-4^+]$	(67.6 × 16.2) × (16.1 × 3.7)	$\left(\frac{3^+}{4^+}\right) \times \left(\frac{2^+}{4^+}\right)$	18	26	28		24		1:1:1:1	2.33 NS

C Testing linkage between anodic locus 1 and locus 2, 3, 4

Cross	Genotypes suggested	Segregations observed for anodic peroxidase phenotypes				Theoretical segregation	χ^2 value
		$[1^+3^+4^+]$	$[1^-3^+4^+]$	$[1^+3^+4^-]$	$[1^-3^+4^-]$		
(67.5) × (67.5 × 16.6) $[1^-3^+4^-] \times [1^+3^+4^+]$	$\left(\frac{1^-\ 3^+}{1^-\ 3^+}\right) \times \left(\frac{1^+\ 3^+}{1^-\ 4^+}\right)$	20	25	22	33	1:1:1:1	3.52 NS

[a] Cross between 8.6 and one of its self-progenies, 94, with a phenotype $[1^-]$
[b] Bud pollination
NS Non significant at 0.05 level

Table 3. Determination of cathodic isoperoxidase genotypes

Parent plant tested			Test crosses	Segregations observed of isoperoxidase I, II phenotypes			Cathodic peroxidase genotype of parent tested
No.	S genotype	Cathodic peroxidase phenotypes		$[I^- II^+]$	$[I^+ II^-]$	$[I^+ II^+]$	
N. langsdorffii							
3.7	$S_c S_c$	$[I^- II^+]$	Selfing 3.7	24	0	0	$\frac{I^- II^+}{I^- II^+}$
			16.1 × 3.7	0	0	65	
N. alata							
67.6	$S_{F10} S_x$	$[I^- II^+]$	Selfing[a] 6.76	42	0	0	$\frac{I^- II^+}{I^- II^+}$
			3.7 × 67.6	30	0	0	
16.2	$S_a S_a$	$[I^- II^+]$	67.6 × 16.2	30	0	0	$\frac{I^- II^+}{I^- II^+}$
16.1	$S_e S_e$	$[I^+ II^-]$	Selfing[a] 16.1	0	18	0	$\frac{I^+ II^-}{I^+ II^-}$
16.6	$S_a S_a$	$[I^+ II^-]$	16.6 × 16.1	0	47	0	$\frac{I^+ II^-}{I^+ II^-}$
			16.6 × 3.7[b]	0	0	55	
67.5	$S_{F10} S_{F11}$	$[I^+ II^+]$	3.7 × 67.5, 67.5 × 67.6, 67.6 × 16.2	40	0	50	$\frac{I^+ II^+}{I^- II^+}$
			67.5 × 16.6, 68.8 × 67.5	0	0	50	
67.8	$S_{F10} S_{F11}$	$[I^+ II^+]$	67.8 × 67.6	6	0	9	$\frac{I^+ II^+}{I^- II^-}$
			67.8 × 16.1 / 16.6	0	17	21	
68.7	$S_1 S_1$	$[I^+ II^+]$	68.7 × 67.6[b]	0	0	30	$\frac{I^+ II^+}{I^+ II^-}$
			68.7 × 16.1	0	14	16	
68.8	$S_1 S_3$	$[I^+ II^+]$	Selfing[a] 68.8	0	5	10	$\frac{I^+ II^+}{I^+ II^-}$
			68.8 × 67.6 / 67.5	0	0	45	
8.6	$S'_a S'_y$	$[I^+ II^+]$	Selfing 8.6	0	6	9	$\frac{I^+ II^+}{I^+ II^-}$
			× $[I^+ II^-]$[c]	0	33	47	

[a] Bud pollination
[b] Both direct and reciprocal crosses
[c] A self-progeny from 8.6

linkage between S-locus and the gene involved in the expression of isozyme P_1.

b) Isozymes 2, 3, and 4 (Table 2B). The results of selfings and one cross heterozygous for the combination of these three bands fit with the assumption that there are three allelic forms at the same locus. All alternative hypotheses (two gene models) were ruled out because a maximum of 3 phenotypes was observed in the self-progenies and only 4 phenotypes in a $[3^+4^+] \times [2^+4^+]$ cross. The test-cross of $(1^-1^-3^+3^+) \times (1^+1^-3^+4^+)$ (Table 2C) yields a [1:1:1:1] ratio, which indicates that the isozyme P_1 gene is a distinct and independent locus from the $P_{2,3,4}$ gene. As all selfs and crosses gave the same segregation ratios for the isozyme bands, we can conclude that the P_1 and $P_{2,3,4}$ loci are not linked to the S-locus.

Inheritance of cathodic peroxidases

Over all, two cathodic peroxidase bands (P_I and P_{II}) were observed in this study. The results of our genetic studies (Table 3) were in perfect agreement with a one gene hypothesis for each band with two alleles. The presence of each band is dominant over its absence.

Table 3 summarizes the progeny involved, the phenotype and the genotype of each parent.

a) Linkage tests between S and P_I. Two test-crosses between $♀\frac{P_I^-}{P_I^-} \times ♂\frac{P_I^+}{P_I^-}$ genotypes demonstrate a very high distortion from the expected $1[P_I^+]:1[P_I^-]$ ratios (Table 4), which indicates that the compatible S_{F11} allele is closely linked to the P_I^+ allele. Also a $♀\frac{I^- S_{F10}}{I^+ S_{F11}} \times ♂\frac{I^- S_a}{I^- S_a}$ cross produces only two recombinant plants $\left(\frac{S_{F10}}{S_a}, \frac{P_I^+}{P_I^+}\right)$ in the 60 progenies tested for the S and P_I alleles.

The above results clearly confirm that the S_{F11} and the P_I^+ alleles are located on the same chromosome. The distance between these two loci (S and P_I) is estimated as $r_I = 2.8$ cM with a 0.95 confidence interval (1.4–5.7).

b) Linkage tests between S and P_{II}. The test-cross $\frac{S_e P_{II}^-}{S_e P_{II}^-} \times \frac{S_I P_{II}^+}{S_e P_{II}^-}$ gave only 32 plants of $[P_{II}^-]$ phenotype among 100 progeny plants tested (instead of 1:1 ratio) (Table 5). S_I allele seems the to be linked to the P_{II}^+ with a recombination rate of 32 cM.

Two other crosses have results in agreement to such a linkage. The pooled values indicate that $r_{II} = 34 \pm 6.6$ cM. In another experiment, in which a com-

Table 4. Crosses and segregations used to estimate the linkage between S and P_I (all plants are $\left(\frac{II^+}{II^+}\right)$ for P_{II})

Parents		Segregation of isoperoxidase I phenotype		χ^2 test 1:1	r(cM)
♀	♂	$[I^+]$	$[I^-]$		
67.6	67.5				
$\frac{I^- S_{F10}}{I^- S_x} \times$	$\frac{I^+ S_{F10}}{I^- S_{F11}}$	48	2	42.32**	4[a]
67.6	67.6 ×67.5[c]				
$\frac{I^- S_{F10}}{I^- S_x} \times$	$\frac{I^+ S_{F11}}{I^- S_x}$	137	3	128.25**	2[a]
67.5	16.2				
$\frac{I^+ S_{F10}}{I^- S_{F11}} \times$	$\frac{I^- S_a}{I^- S_a}$	$\frac{S_{F11}}{S_a}$ 0 ; $\frac{S_{F10}}{S_a}$ 35 } 35	$\frac{S_{F11}}{S_a}$ 23 ; $\frac{S_{F10}}{S_a}$ 2 } 25	1.67	3[b]

[a] r is calculated on the assumption that segregation distortions are entirely due to the linkage between S and P_I
[b] r calculated after complete analysis identifying incompatibility among progeny: only 2 recombinations observed among 60 plants
[c] Genotype ascertained by specific test of S-incompatibility; progeny of the first cross described in the table
** Significant at 0.01 level

Table 5. Crosses and segregations used to estimate the linkage between S and P_{II} (all plants are $\frac{I^+}{I^+}$ for P_I)

Parents		Segregations of peroxidase II phenotypes				χ^2 test	r(cM)
♀	♂	$[II^+]$		$[II^-]$			
16.1	68.7 × 16.1					1:1	
$\left(\frac{S_c I^-}{S_c II^-}\right)$	$\left(\frac{S_I I^+}{S_c II^-}\right)$	68		32		12.96**	32[a]
68.7	68.8					3:1	
$\left(\frac{S_I II^+}{S_I II^-}\right)$	$\left(\frac{S_I II^+}{S_3 II^-}\right)$	82		18		2.6 NS	36[b]
d	8.6	$[S'_a II^+]$	$[S'_a II^-]$	$[S'_a S'_y II^+]$	$[S'_a S'_y II^-]$	1:1 $\frac{S_a}{S_x}$ 0.05 NS	
$\left(\frac{S'^-_a}{S'^-_a}\right)$	$\left(\frac{S'_a II^+}{S'_y II^-}\right)$	31	8	16	25	1:1 $\frac{II^-}{II^+}$ 2.45 NS linkage: 12.8**	30[c]

[a] r is calculated on the assumption that segregation distortions are entirely due to the linkage between S and P_{II}
[b] r is calculated despite the non-significant χ^2 for comparison with the other estimations
[c] r is calculated after complete analysis identifying incompatibility among progeny
[d] Offspring of a 8.6 selfing
NS Non significant at 0.05 level
** Significant at 0.01 level

plete analysis was performed, S. Hamon (unpublished) found a similar value $r_{II} = 30 \pm 10$ cM.

c) Simultaneous linkage tests between S, P_I and P_{II}. Two crosses between $\frac{P_I^+ P_{II}^-}{P_I^+ P_{II}^-} \times \frac{P_I P_{II}^+}{P_I^+ P_{II}^-}$ genotypes (Table 6) demonstrated a very close linkage between the S and P_{II} loci ($r_{II} = 1$ cM), in sharp contrast to the preceding data $r_{II} = 34$ cM (Table 5). In the self-progeny of double heterozygous $\frac{P_I^+ P_{II}^-}{P_I^- P_{II}^+}$ it was also found that $S - P_I$ distance was much longer (17–19 cM instead of 2.8 cM estimated in Table 4).

There are seemingly conflicting estimation of $S - P_I$ and $S - P_{II}$ distances from Tables 4, 5, and 6. One could point out that, in one cross and in all 3 self-progenies of Table 6, a *N. langsdorffii* parent (3.7 plant) background was involved. In interspecific crosses there might occur another S-bearing chromosome rearrangement which should explain the reversed $S - P_{II} - P_I$ mapping rather than $S - P_I - P_{II}$ (the order of loci is arbitrary in this writing) in intra specific *N. alata* crosses. However this assumption does not explain the result of the second *N. alata* × *N. alata* cross [16.6 × (67.5 × 16.6)] which indicated also $r_{II} = 1$ cM.

Pollen tube certation cannot explain the results because we observed, Table 4, the same results either after bud pollination of self incompatible plants or after normal selfing of compatible plants. Moreover the whole set of data gave quantitatively coherent results for segregations and linkage relationships.

A second explanation is based in the fact that we never observed the $P_I^- P_{II}^-$ phenotype as expected in the self-progeny of $\frac{P_I^+ P_{II}^-}{P_I^- P_{II}^+}$ genotype. We calculated the expected data (in Table 6) with the assumption that the $P_I^- P_{II}^-$ gametes (both in ♀ and ♂ gamete populations) do not contribute to the offspring of the next generation. Under this hypothesis, we applied the r values calculated in Tables 4 and 5 ($r_I = 2.8$ cM and $r_{II} = 34$ cM). As illustrated in Table 6, the calculated data fit well with the observed segregations ratios.

Tests for the absence of $[P_I^- P_{II}^-]$ phenotype. Further tests for the absence of $P_I^- P_{II}^-$ gametes and $[P_I^- P_{II}^-]$ phenotype in some appropriate genetic backgrounds were performed (Table 7). In the ♀ $\frac{P_I^- S_c P_{II}^+}{P_I^+ S_e P_{II}^-} \times$ ♂ $\frac{P_I^+ S_e P_{II}^-}{P_I^+ S_c P_{II}^-}$ cross, if ♀ $P_I^- P_{II}^-$ gametes were viable, a $1[I^+ II^+] : 1[I^+ II^-]$ ratio was expected, but the observed data is 61 : 38 ($\chi^2 = 5.34$). The theoretical data calculated with the assumption that ♀ $P_I^- P_{II}^-$ gametes are not viable (with $r_I = 2.8$ cM and $r_{II} = 34$ cM), fit well with the observed data ($\chi^2 = 0.015$). If ♀ $P_I^- P_{II}^-$ gametes were viable among the $[P_I^+ P_{II}^-]$ phenotypes, 21% of such plants should be $P_I^+ P_I^-$. None of the 24 such plants tested were heterozygous for the P_I locus suggesting that ♀ $P_I^- P_{II}^-$ gametes did not yield progenies. The progeny of two other crosses were in accordance with such a ♀ $P_I^- P_{II}^-$ gametes-absence hypothesis.

Also the self-progeny of a $\frac{P_I^- S_c P_{II}^+}{P_I^+ S_c P_{II}^-}$ genotype was in agreement with the absence of ♀ and ♂ $P_I^- P_{II}^-$ gametes.

Table 6. Crosses using double heterozygotes for genes coding cathodic isoperoxidases I and II

Parents		a			b		c		
♀	♂	$[I^+ I^+]$	$[I^- II^+]$	$[I^+ II^-]$	r_I	r_{II}	$[I^+ II^+]$	$[I^- II^+]$	$[I^- II^+]$
16.1	16.1 × 3.7								
$\frac{I^+ S_e II^-}{I^+ S_c II^-}$	$\times \frac{I^- S_c II^+}{I^+ S_e II^-}$	184	0	2		1	183	0	3
16.6	67.5 × 16.6								
$\frac{I^+ S_a II^-}{I^+ S_a II^-}$	$\frac{I^+ S_a II^-}{I^- S_F II^+}$	98	0	1		1	98.5	0	0.5
(16.1 × 3.7) selfed									
$\frac{I^- S_c II^+}{I^+ S_e II^-}$		90	62	1	19	1	95	57	1
(3.7 × 16.6) selfed									
$\frac{I^- S_c II^+}{I^+ S_a II^-}$		69	51	0	17	1	74	45	1

[a] Segregations observed of isoperoxidase I, II phenotypes

[b] r_I and r_{II} values calculated from the observed segregations, reserving the possibility that $(I^- II^-)$ gametes might contribute to offspring

[c] Theoretical segregations calculated using the r values from Tables 5 and 6, under the hypothesis that $(I^- II^-)$ gametes do not contribute to offspring

Table 7. Complementary tests for the absence of $P_I^- P_{II}^-$ gametes (both ♂ and ♀)

Crosses	Observed segregation				A Expected segregation with I^-II^- gametes viable				B Expected segregation with I^-II^- gametes nonviable				χ^2 a	χ^2 b
	$[I^+II^+]$	$[I^-II^+]$	$[I^+II^-]$	$[I^-II^-]$	$[I^+II^+]$	$[I^-II^+]$	$[I^+II^-]$	$[I^-II^-]$	$[I^+II^+]$	$[I^-II^+]$	$[I^+II^-]$	$[I^-II^-]$		
(16.1 × 3.7) × b														
$\left(\frac{I^-S_cII^+}{I^+S_cII^-}\right) \times \left(\frac{I^+S_eII^-}{I^+S_cII^-}\right)$	61		38		49.5		49.5		60.4		38.6		5.34**	0.02 NS
(16.1 × 3.7) × 3.7														
$\left(\frac{I^-S_cII^+}{I^+S_cII^-}\right) \times \left(\frac{I^-S_cII^+}{I^-S_cII^+}\right)$	113		85		9		99		120		78		3.96**	1.04 NS
67.8 × 67.6														
$\left(\frac{I^-S_{F10}II^+}{I^-S_{F11}II^-}\right) \times \left(\frac{I^-S_{F10}II^+}{I^-S_xII^+}\right)$	107		58		132.5		132.5		100		65		14.55**	1.24
Selfing 473.77 a														
$\left(\frac{I^-S_cII^+}{I^+S_cII^-}\right)$	70	10	12		48.5	20.3	20.3	2.9	63.4	14.3	14.3	0	19.62**	2.35 NS
With the following (2/4)	37	4	6	47										
distribution in (2/2)	13	4	3	20	χ^2 2:1:1 = 0.59 NS									
anodic isozymes (4/4)	20	2	3	25										

[a] This plant comes from the following progenies
16.1 × 3.7 → 129
16.1 × 129 → 433.7
129 × 433.7 → 473.77

[b] Plant progeny from (16.1 × 3.7) selfed

** Significant at 0.01 level

The latter selfed plant was heterozygous for one anodic locus $[P_2P_4]$. These bands segregated in a $1[P_2P_2]:2[P_2P_4]:1[P_4P_4]$ ratio. They also segregated independently from the P_I and P_{II} bands (Table 7), which indicates the effective independence between the cathodic and the anodic locus.

The $P_I^- P_{II}^-$ gametes could be eliminated during gametogenesis, or rejected in the fecundation process or could block the germination of seeds. Our tests on aborted pollen rates, the number of seeds per capsule and the % of germinated seeds did not give clear-cut results between potentially $P_I^- P_{II}^-$ producing cross and other cross- or self-progenies. We could not, until now, detect the time of elimination of $P_I^- P_{II}^-$ gametes. This problem is under study in our laboratory.

Thus the hypothesis of missing $[I^-II^-]$ gametes seems to be reliable; over 3,000 plants analysed so far, we never observe any plants with a $[P_I^- P_{II}^-]$ zymogram.

Discussion

Our data demonstrate clearly that for the presence of two cathodic peroxidases two genes are responsibles, located in the S bearing chromosome in *N. alata* and in *N. langsdorffii*. This study seems to be the first report on linkage to the S-locus in these species. The distance $r_I = 2.8$ cM, $r_{II} = 34$ cM seems to be correct but we cannot yet describe the relative position of $S P_I P_{II}$ loci.

It may be possible that the style peroxidase patterns correlated to the S-genotypes, as described by Pandey (1967), included the P_I and P_{II} cathodic bands linked to the S-locus.

The zymogram observed in *N. sylvestris* (D. Prat et al. in preparation) under the same conditions is very distinct from those observed in the *N. alata, N. langsdorffii* group: the number of bands is higher in *N. sylvestris* (6 cathodic bands) and there is no evidence of homology. In *N. sylvestris*, mendelian analysis was impossible because there was no polymorphism observed in the collection.

In *N. tabacum*, Mäder (1976) demonstrated there were several groups of peroxidase isoenzymes, with different primary structures and different localizations (cell wall or cytoplasmic). Thus they depend on several different genes but we cannot compare these isoenzymes with those studied in the present paper.

In conclusion, the present analsyis has permitted the finding of two marker genes for the S linkage group and the explaining of some of the convergences noted between the S genotypes and peroxidase phenotypes. Given the difficulties encountered in interpreting the

data issuing from the crosses, it is not surprising that this Mendelian linkage has not before been demonstrated. Clearly it would be interesting to test if the stylar isoperoxidase genes studied by Pandey (1967) and Bredemeijer and Blaas (1981) are the same as those studied here and whether or not they are linked to S. These marker genes have already been used to analyze segregation distortions following androgenesis (Kheyr-Pour et al. 1982) and in plants regenerated by tissue culture (Prat, unpublished).

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