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Do S Allele-specific Peroxidase Isoenzymes Exist in Self-incompatible *Nicotiana alata*?

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Summary: In order to test Pandey's hypothesis that peroxidase isoenzymes determine S-gene specificity in *Nicotiana alata*, peroxidase isoenzymes in styles and pollen from various plants of an inbred- and a cross progeny were compared by means of starch gel electrophoresis and electrofocusing.

No relation between the S-genotype and the peroxidase isoenzyme patterns of pollen or of styles could be established. The differences between the isoenzyme patterns of different S-genotypes were ascribed to differences in the genetic background of various plants that had the same S-genotype.

Key words: Self-incompatibility – *Nicotiana alata* – S Alleles-peroxidase isoenzymes-Styles – Pollen

Introduction

After Pandey's report (Pandey 1967) that peroxidase isoenzymes determine S-gene specificity in *Nicotiana alata* several authors have carried out investigations on other self-incompatible species to study the relation between S-genotype and peroxidase isoenzyme pattern (for a review, see de Nettancourt 1977).

Nasrallah et al. (1970) and Nishio and Hinata (1977) have tried in *Brassica oleracea* to detect a relation between the peroxidase isoenzyme pattern of the stigma and the S-genotype, but without success. They found that the S-specific proteins in the stigmas of *Brassica* exhibited no peroxidase activity. In *Lilium longiflorum* no apparent association was observed between the self-incompatibility reaction and peroxidases (Desborough and Peloquin 1968). Attempts to identify S-genotypes from vegetative tissues by comparing total peroxidase activity in leaves of *Trifolium pratense* (Anderson et al. 1974) and peroxidase isoenzymes in leaves of *Lycopersicum peruvianum* (Bredemeijer 1977) were unsuccessful.

In view of the fact that the data reported by various authors on the relation between peroxidase isoenzyme patterns and S-genotypes in different species were contradictory, a confirmation of the results in *N. alata* seemed necessary. It is also important for our studies on the role of peroxidase in the rejection of incompatible pollen tubes to ascertain if S-specific peroxidases exist in the clones of *N. alata* used at our laboratory.

Materials and Methods

The two self-incompatible clones of *Nicotiana alata* Link and Otto, namely OWL (S_2S_3) and OB-2 (S_6S_7) described by Carluccio et al. (1974) were used. Plants of clone OWL were bud-pollinated or crossed with clone OB-2. The S-genotypes of the resulting progenies were determined by crossing with tester-stocks. For each S-genotype at least five plants were used for studying the peroxidase isoenzyme patterns. Flowers used for analysis were harvested during the day after anthesis from plants grown in a greenhouse (natural light; night temperature 18°C, day temperature 24-34°C). In one series of experiments styles were immediately used for the extraction of peroxidases, whereas in another series flowers were cross-pollinated and incubated during 7 days at 15°C (8,000 lux, 16 h; darkness 8 h) before extracting the stylar peroxidases. Style extracts were prepared by homogenizing 14 styles with 1 ml 4% NaCl solution in an icecooled mortar. The supernatants obtained after centrifugation for 45 min at 18,000 g were concentrated 5 times in a Minicon A-25 concentrator. Pollen extracts were prepared by homogenizing 50 mg pollen in an ice-cooled Potter Elvehjem homogenizer with 1.1 ml 4% NaCl solution. Supernatants (18,000 g, 45 min) were concentrated twice. The starch gel electrophoresis and staining for peroxidase with benzidine were carried out as described earlier (Bredemeijer 1974). Isoelectric focusing was performed on a prepared thin-layer polyacrylamide gel plate containing Ampholine carrier ampholytes in the pH range 3.5-9.5 (L.K.B., Sweden). Samples of 20 µl of extracts dialyzed against 0.05 M phosphate buffer pH 7.0 were applied to pieces of filter paper (5 × 10 mm) which were placed on the gel surface. After focusing, the gels were incubated for 1 h in a solution containing 0.1 g 0-dianisidine dissolved in 5 ml ethanol, 95 ml 0.2 M sodium acetate buffer, pH 5.0, and 2 ml 3% hydrogen peroxide to stain the peroxidases.

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Results

1 Peroxidase Isoenzyme Patterns of Unpollinated Styles

Peroxidase isoenzymes were analysed by three different methods, namely acrylamide gel block electrophoresis as described by Pandey (1967), starch gel electrophoresis and electrofocusing. The first method gave rise to separations with several faint or wide bands causing difficulties in comparing the isoenzyme patterns. Therefore, the results obtained by this method are not presented here. However, no relation was observed between S-genotype and peroxidase isoenzyme pattern.

With starch gel electrophoresis the peroxidase bands were fairly well separated, and the location of each band could be compared between the S-genotypes. The peroxidase isoenzymes of the two parental clones, OWL and OB-2 and their F_1 progeny that differ in S-genotype are shown in Fig. 1. Whereas the bands 7 and 12 were characteristic of OWL, the bands 13 and 16a were associated with OB-2. The F_1 progeny was characterized by the presence of bands 12, 13, 16a and 7 or 8. The other bands, namely 10, 15, 18 and 19 were present in the parent clones and their F_1 progeny, but the staining intensity was higher in OB-2 than in OWL. The progeny showed an intermediate staining intensity. The results clearly indicate that the differences between the isoenzyme patterns of different S-genotypes, as shown in Figure 1, could be ascribed to differences in the genetic background of various plants that had the same S-genotype: the plants showed differences both in quality, i.e. occurrence of bands, and staining intensity of the particular bands (Fig. 2).

Figure 3 shows the banding patterns of the progeny

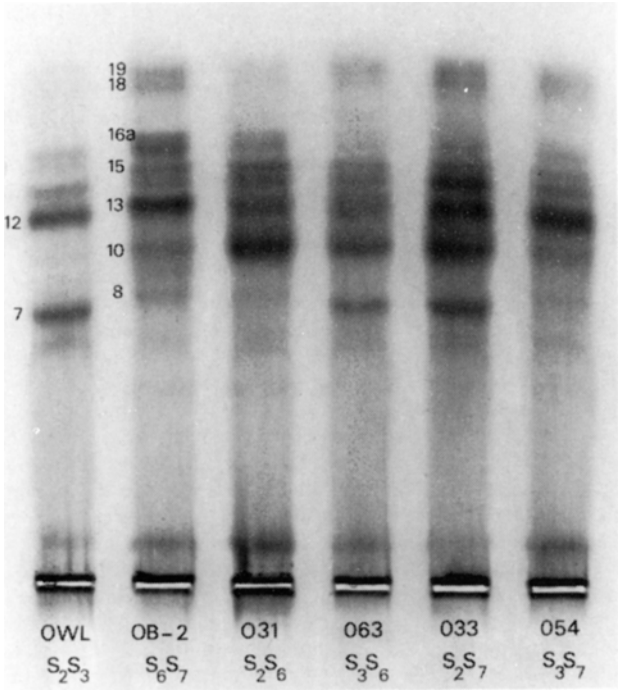

Fig. 1. Starch gel electrophoresis of peroxidase isoenzymes in the styles of the parent clones OWL-S_2S_3 and OB-2-S_6S_7 and their F_1 progeny

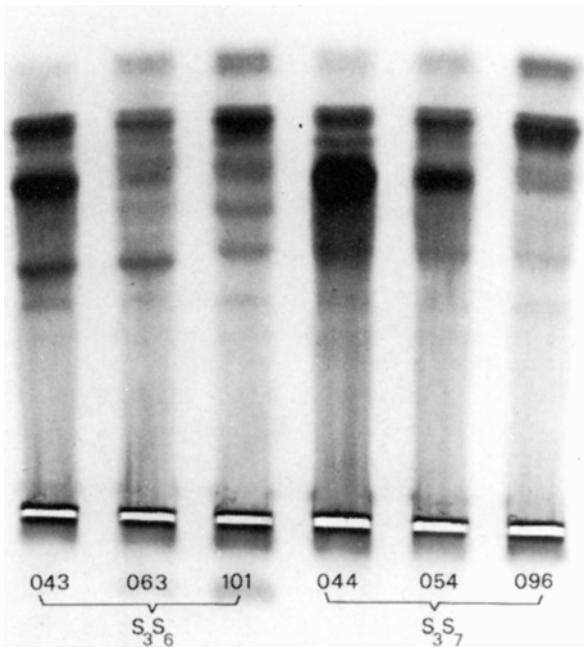

Fig. 2. Peroxidase isoenzymes in the styles of different plants of S_3S_6 and S_3S_7

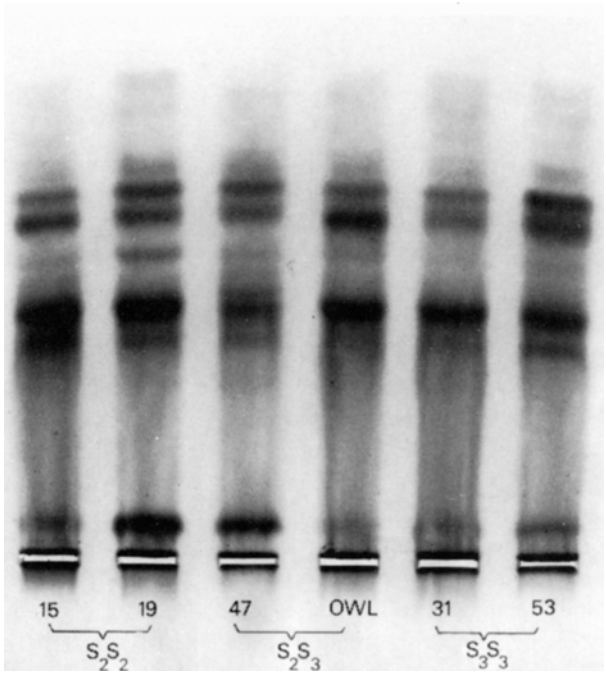

Fig. 3. Stylar peroxidase isoenzymes of different S-genotypes among the inbred progeny of OWL-S_2S_3

derived from Qbud-pollination of OWL (S_2S_3). These results show that there were neither S-specific bands nor S-specific combinations of bands, and thus confirm the results on F_1 progeny.

Isoelectric focusing of the peroxidase isoenzymes was tried but with varying success. The staining intensity of the bands showed variations, probably caused by endogenous phenolic inhibitors which migrate together with the isoenzymes (Legrand et al. 1976). The results obtained with this method agree closely with those obtained by starch gel electrophoresis. The peroxidase isoenzyme patterns were independent of the S-genotypes (Fig. 4). Differences between the patterns were due to variation of the bands among individuals within each S-genotype.

2 Peroxidase Isoenzyme Patterns of Cross-pollinated Styles

Since in cross-pollinated styles after fertilization the activity of peroxidase isoenzymes greatly increases when compared with that in unpollinated styles, a more precise comparison of the banding patterns was possible.

Although the increase in activity of particular peroxidase isoenzymes was not the same in all plants, a qualitative comparison of the patterns was well possible. As can be seen in Figure 5, the patterns of cross-pollinated styles mainly exhibited easily detectable bands, in contrast to the unpollinated styles, where the patterns showed several weakly stained bands (Figs. 1 and 3). It is clear from the banding patterns (Fig. 5) that there were no discernable differences between the S-genotypes.

3 Peroxidase Isoenzyme Patterns of Pollen

Peroxidase isoenzyme polymorphism in pollen was low when compared with that in the style. Most of the plants of inbred- and cross progenies had only 4 to 5 pollen peroxidases.

The isoenzyme patterns of the two parental clones OWL and OB-2 could easily be distinguished. The bands 4 and 6 were associated with OWL, while band 5 was characteristic of OB-2 (Fig. 6). Further, the OWL pattern exhibited a darkly stained band 7, in contrast to the OB-2 pattern, where only a lightly stained band 7 was observed. All the F_1 progenies showed either the bands 4 and 6 or band 5. Band 7 always had an intermediate staining intensity.

A comparison of the isoenzyme patterns of the various plants among the inbred progeny (Fig. 7) and F_1 progeny (Fig. 6) revealed that there was no relation between the peroxidase isoenzyme pattern and the S-genotype of pollen and that the differences between the banding patterns could be ascribed to variations in genetic background of the progenies.

Discussion

The results obtained in the present study by using several extraction methods and types of electrophoresis do not

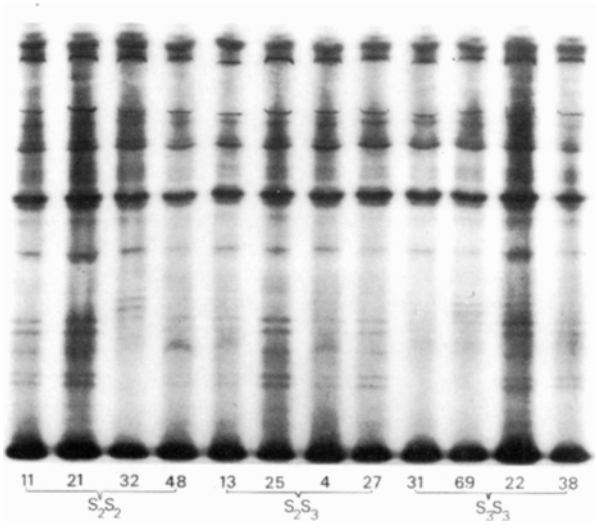

Fig. 4. Isoelectric focusing of peroxidase isoenzymes in styles of the inbred progeny of OWL-S_2S_3

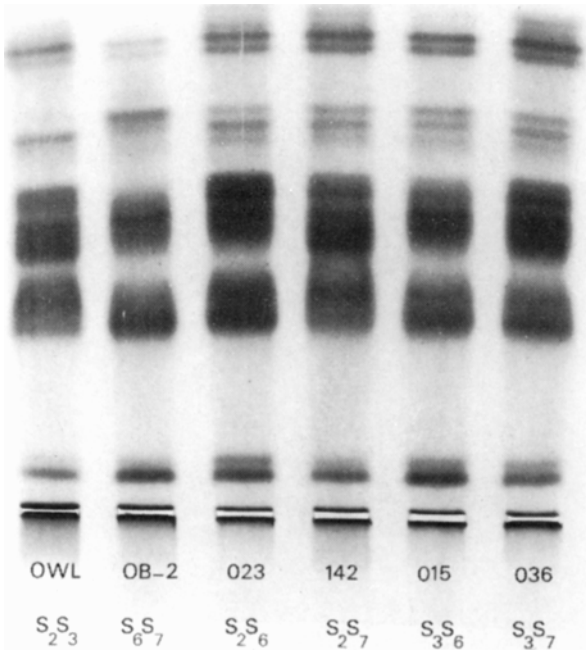

Fig. 5. Starch gel electrophoresis of peroxidase isoenzymes in cross-pollinated styles of OWL, OB-2 and the F_1 progeny

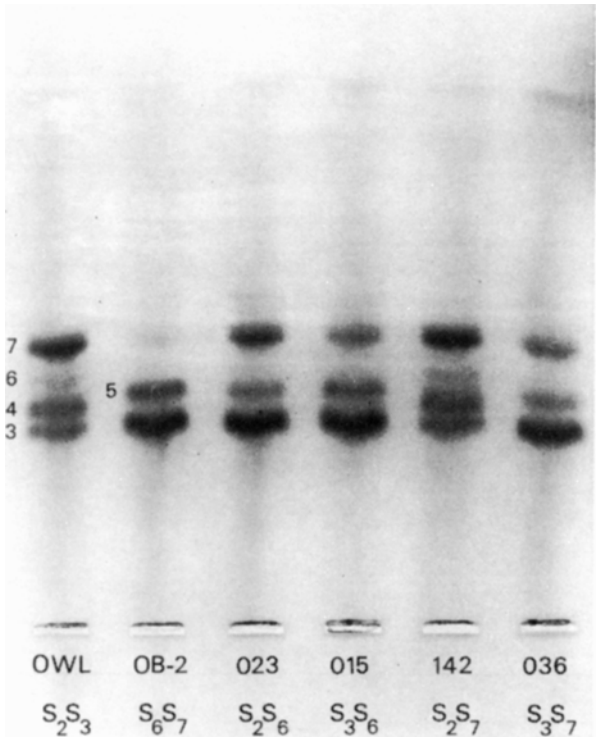

Fig. 6. Starch gel showing pollen peroxidase isoenzymes in OWL, OB-2 and their F_1 progeny

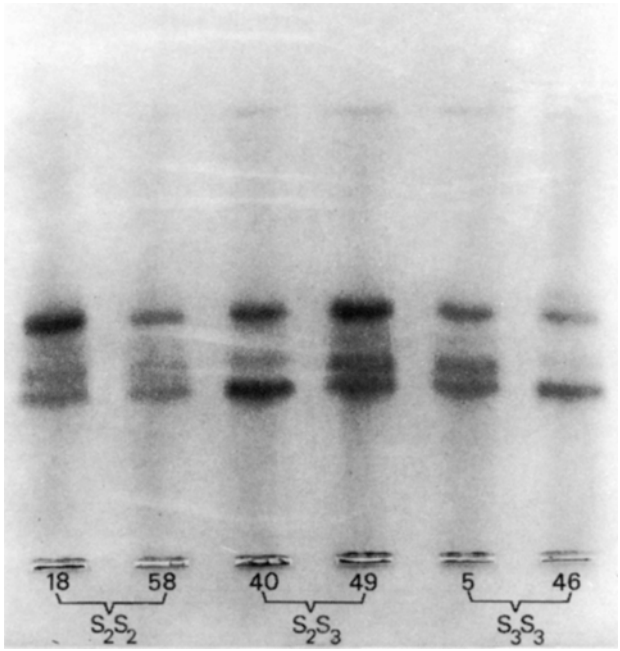

Fig. 7. Pollen peroxidase isoenzymes in the inbred progeny of OWL

confirm the claim (Pandey 1967) that each S-allele in *N. alata* styles has its own specific peroxidase isoenzyme bands. Neither in unpollinated nor in cross-pollinated styles could any relation between S-genotype and peroxidase isoenzyme pattern be established. It was also evident that the peroxidase isoenzyme patterns of pollen were independent of the S-genotype. The differences between the isoenzyme patterns of different S-genotypes were ascribed to differences in the genetic background of various plants that had the same S-genotype.

The discrepancy between Pandey's results and those presented in this study might be explained by assuming that the relation between S-genotype and peroxidase isoenzyme pattern as found by Pandey (1967) was a nonspecific one, that is, that the peroxidases concerned were coded by closely linked genes instead of by the S-locus. Probably, this possibility remained unnoticed by Pandey as the experiments were performed with relatively few plants. It is clear that in our plant material such a nonspecific relation between S-genotype and peroxidase isoenzyme pattern did not exist.

The demonstration that no S specific peroxidases exist in *N. alata* however does not rule out the possibility of the involvement of peroxidase as a nonspecific component of the incompatibility reaction or as one of the physiological characters of a style that influences the pollen tube growth. Previously, it has been shown that peroxidase is able to inhibit the growth of *N. alata* pollen tubes *in vitro* (Bredemeijer 1975). Furthermore, one of the peroxidase isoenzymes in *N. alata* styles does indeed appear to influence the growth of incompatible pollen tubes, as a factor besides the incompatibility reaction itself (Bredemeijer 1978, 1979).

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