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S-Specific Proteins in Styles of Self-incompatible *Nicotiana alata*

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Summary. A comparison of the stigma protein patterns of individual plants of the inbred- and cross-progenies in *Nicotiana alata* by isoelectric focusing revealed the presence of S-specific proteins. The S allele-protein relationship was found for three different S alleles. The S-specific proteins occurred in both stigma and stylar parts of the pistil whereas they were absent in leaves. In clone OWL the concentration of S-specific proteins in the stigma increased gradually during floral development. The shift from compatibility to incompatibility was not accompanied by an abrupt increase in concentration of the S-proteins.

Key words: Self incompatibility – *Nicotiana alata* – S allele specific proteins – Styles

Introduction

The model for the control of gametophytic incompatibility proposed by Van der Donk (1975) for *Petunia* includes only post-pollination synthesis of the S-specific stylar recognition proteins. In contrast, serologically detected S-allele specific antigens were found in the transmitting tissue of unpollinated *Petunia* styles by Linskens (1960). The studies by Gilissen (1978) on the effect of X-rays on pollen tube growth in *Petunia* also suggested that S-specific substances must, at the time of pollen tube growth, be synthesized and present in the style. Further, in *Brassica*, which has a sporophytic self-incompatibility system, S-allele specific proteins have been detected serologically and electrophoretically in unpollinated stigmas (see review in Ferrari and Wallace 1977; Nishio and Hinata 1977, 1978).

In view of the fact that the data with respect to the gametophytic system were contradictory, a re-investigation on the stylar proteins seemed necessary. Therefore, attempts have been made to detect S-specific proteins in unpollinated styles of the test species *Nicotiana alata* by means of isoelectric focusing, the method by which Nishio and Hinata (1977, 1978) were able to detect S-specific proteins in *Brassica* stigmas.

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), and their inbred- and cross-progenies used in the present study were the same as those utilized previously for the comparison of the stylar peroxidase isoenzymes (Bredemeijer and Blaas 1980).

The stigma extracts were prepared by homogenizing the topmost 2 mm of 28 unpollinated styles with 0.65 ml 4% NaCl solution in an ice-cooled mortar. The supernatants obtained after centrifugation for 45 min at 18,000 g were dialysed against 0.05 M phosphate buffer, pH 7.0, and concentrated 10 times in a Minicon A-25 concentrator.

Analytical thin layer gel electrofocusing was performed on prepared polyacrylamide gel plates containing Ampholine carrier ampholytes in the pH range 3.5-9.5 or 5.5-8.5 (L.K.B., Sweden). Fixation and staining with Coomassie Brilliant Blue R250 and destaining were carried out as described in the L.K.B. instructions except that the staining time was 2 hrs instead of 10 min.

Results

1 Protein Patterns of Stigmas in the Inbred Progeny

The stigmatic extracts were subjected to isoelectric focusing in a pH range from 3.5 to 9.5. The patterns revealed many protein bands ranging from pH 3.5 to pH 9.5. Variation of the protein patterns among the individuals of the inbred progeny of clone OWL (S_2S_3) was tested using 7 plants of S_2S_2, 10 of S_2S_3 and 6 of S_3S_3. The band patterns of 6 plants, 2 of each S-genotype, are shown in Figure 1. Pollen protein patterns of the same plants are included for comparison.

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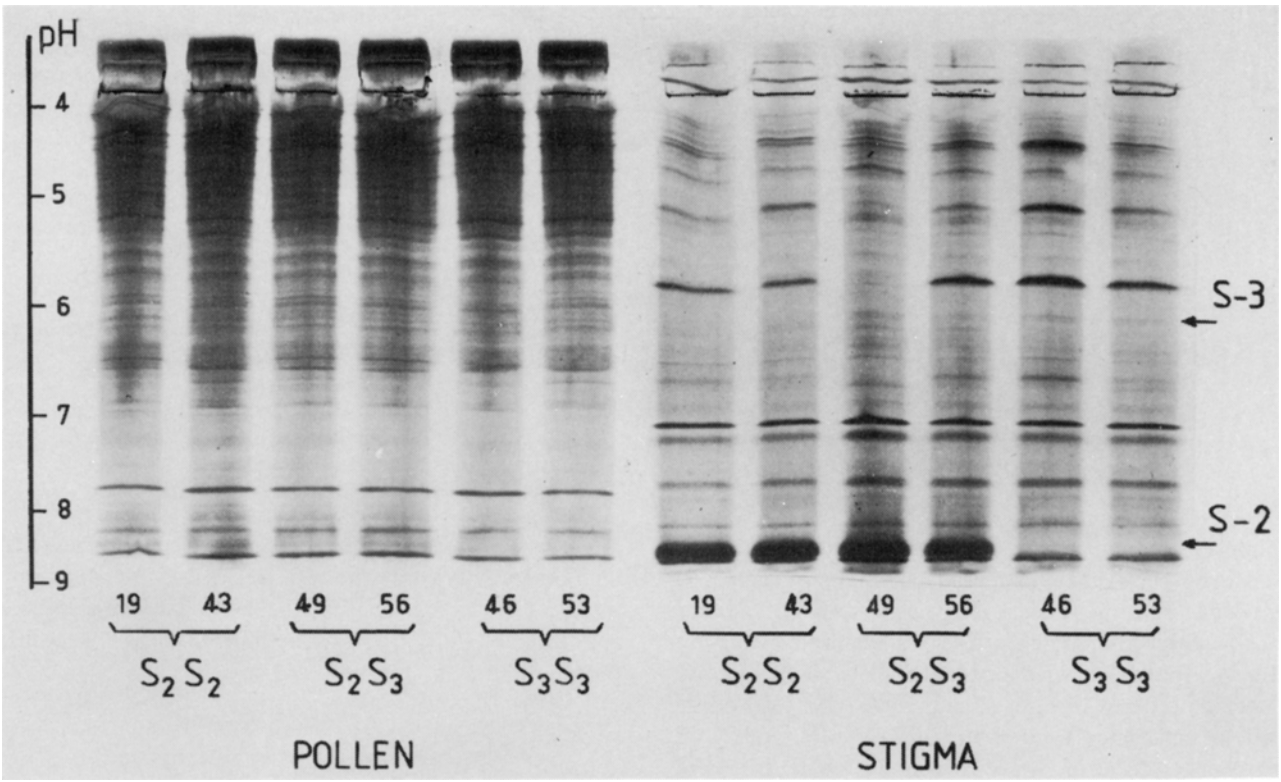

Fig. 1. Electrofocusing of proteins from the pollen- and stigmatic homogenates in various plants of the inbred progeny of OWL (S_2S_3)

A study of the stigma protein patterns revealed the presence of a dense broad band (S-2) focused at a pH of 8.8 in all the genotypes carrying an S_2 allele. This band was absent in the patterns of the S_3S_3 plants examined. On the other hand, the plants carrying the S_3 allele always exhibited a faint narrow band (S-3) at the pH 6.2 region which was absent in S_2S_2 plants. The other differences in band patterns between the various plants were independent of the S-genotype.

The pollen grain extracts yielded a higher number of protein bands than the stigma extracts (Fig. 1). In fact, the number of bands was so high in the former that an accurate comparison of the patterns between different S-genotypes was almost impossible, especially at lower pH regions. Nevertheless, it is clear that the dense S-2 band detected in the stigma patterns at pH 8.8 was not present in the protein patterns of the pollen.

2 *Protein Patterns of Stigmas in the F_1 Plants*

The protein patterns of the two parental clones OWL and OB-2 and their F_1 progeny, 6 plants of each S-genotype, have been analysed by isoelectric focusing at pH gradients from 3.5 to 9.5 and from 5.5 to 8.5. The results obtained with pH gradient 3.5-9.5 are shown in Figure 2.

The distinct band S-2 and the faint narrow band S-3 observed in the protein patterns of the inbred progeny (Fig. 1) also occurred in the F_1 plants carrying the S_2 allele and S_3 allele respectively. Several other differences in the band patterns between OWL and OB-2 segregated independently of the incompatibility genotypes. No specific bands associated with the S_6- or S_7 allele were observed.

As stigma extracts contained numerous proteins it was not excluded that certain protein bands were focused very close to each other due to which an accurate comparison of the bands between S-genotypes was impossible. To separate such bands electrofocusing was carried out at a pH gradient from 5.5 to 8.5 (Fig. 3). At the pH 6.2 region, as on gels of pH 3.5 to 9.5 (Fig. 2), again the band S-3 could be distinguished, but band S-2 (at pH 8.8 in Fig. 2) was not visible anymore. Further comparison of the protein patterns revealed the presence of a faint band (S-6) at the pH 7.8 region in plants carrying the S_6 allele. This band was absent in genotypes that had no S_6 allele. No specific band associated with the S_7 allele could be identified.

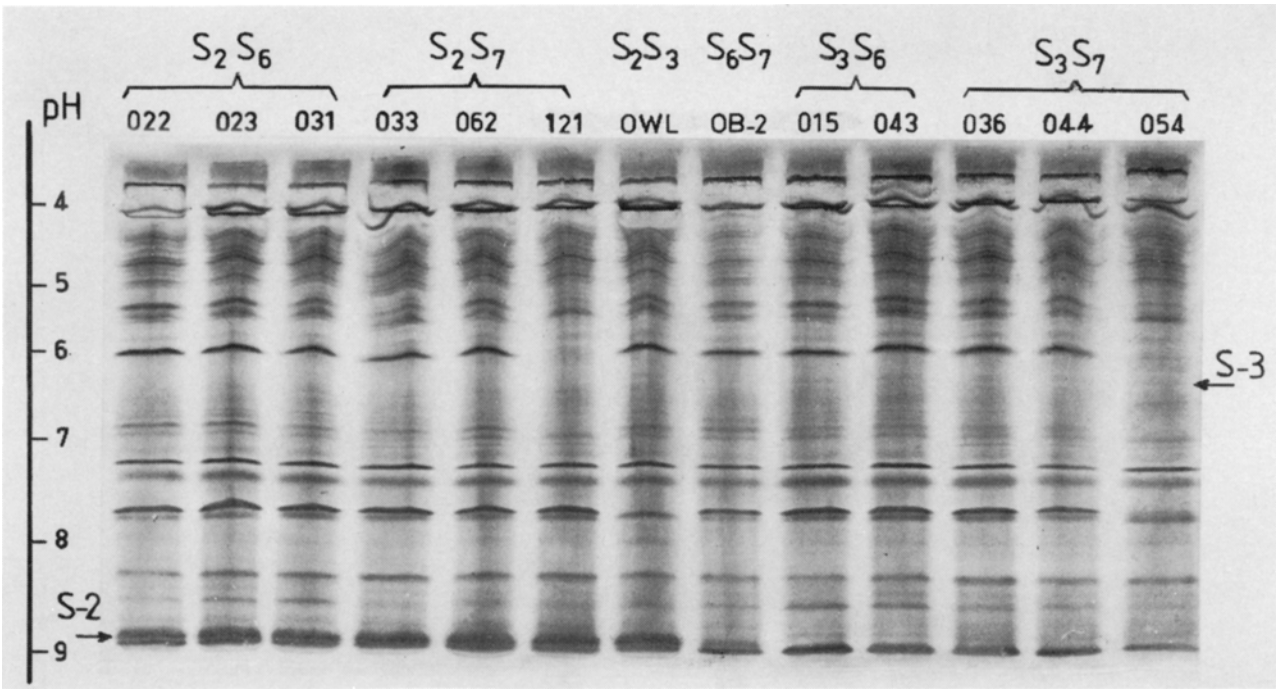

Fig. 2. Electrofocusing of proteins from the stigmatic homogenates of the parent clones OWL (S_2S_3) and OB-2 (S_6S_7) and their F_1 progeny

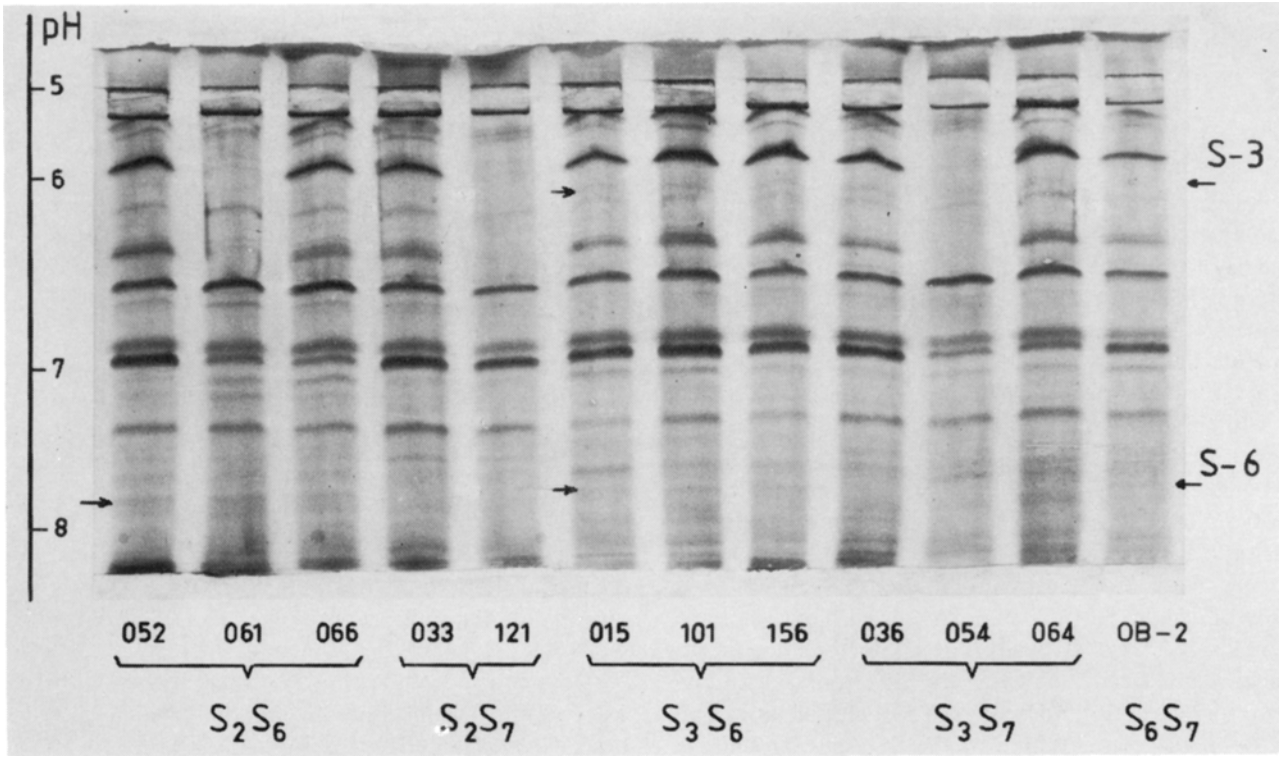

Fig. 3. Electrofocusing in a pH gradient from 5.5 to 8.5 of the stigmatic proteins in the F_1 progeny of OWL X OB-2

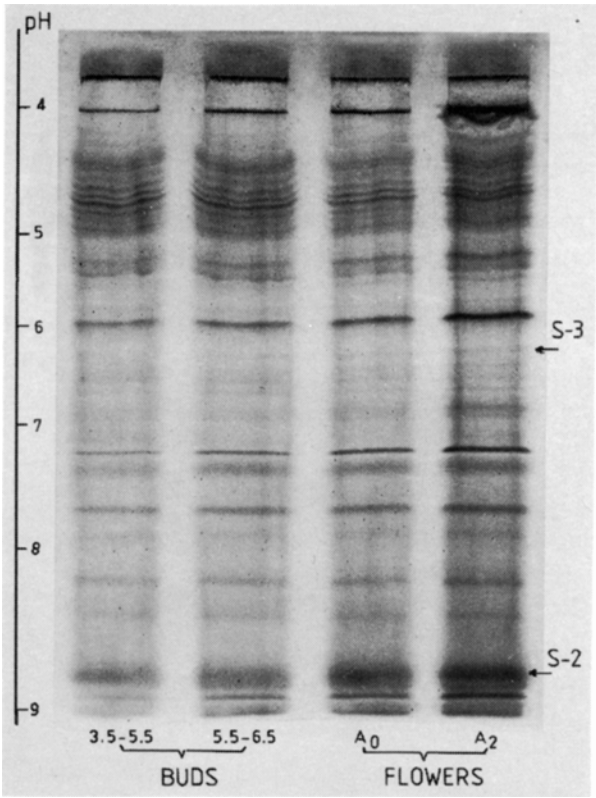

Fig. 4. Protein patterns of the young stigmas from buds of lengths 3.5-5.5 cm and 5.5-6.5 cm and of mature stigmas from flowers at anthesis (A_0) or 2 days after anthesis (A_2) in OWL (S_2S_3)

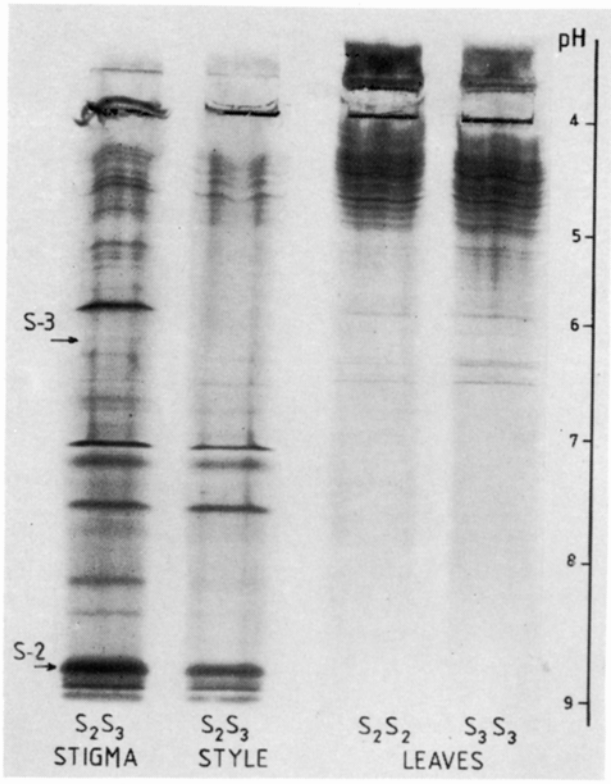

Fig. 5. Protein patterns of the stigma- and stylar part of the pistil (OWL) and of leaves from an S_2S_2 and an S_3S_3 plant. Equal amounts of fresh weight of the various tissues were used for extraction

3 Protein Patterns of Immature Stigmas

In *N. alata* (clone OWL) the shift from compatibility to incompatibility begins in buds of length 5.5-6.5 cm, i.e. one day before anthesis; subsequently the strength of the incompatibility reaction increases over several days. Therefore, an experiment was carried out to determine whether this shift was accompanied by an abrupt increase in intensity of S-specific bands.

Protein patterns of immature and mature stigmas of clone OWL (S_2S_3) were analysed by electrofocusing in a pH gradient from 3.5 to 9.5. 28 Stigmas per sample were analysed in each group. The protein patterns presented in Figure 4 show that the S-2 and S-3 proteins were already present, at relatively low concentrations, in stigmas from buds of length 3.5-5.5 cm. The concentration of these proteins increased gradually during floral development. These results clearly show that the shift from compatibility to incompatibility did not coincide with an abrupt increase in the concentration of the S-specific proteins in the stigma.

4 Protein Patterns of Stigma and Stylar Part of the Pistil

Comparison of the protein patterns of the stigma part and the stylar part of the pistil revealed the presence of S-specific proteins in both parts (Fig. 5). On a fresh weight basis the S-protein concentration was higher in the stigma than in the style.

Further, the analyses show that the greatest quantity of S-specific proteins occurred in the transmitting tissue whereas the cortex contained considerably a lesser quantity. It may be possible that the S-proteins in the cortex extracts originate from the transmitting tissue contaminating the cortex fraction. The fact that the S-specific proteins mainly occur in the transmitting tissue explains why their concentration is relatively high in the stigma; the quantity of transmitting tissue present in the stigma was more or less equal to that in the rest of the style.

The extracts from leaves were also analysed by isoelectric focusing, but no S-specific proteins could be detected (Fig. 5).

Discussion

The results obtained in the present study with isoelectric focusing of the proteins in *N. alata* plants differing in S-genotype clearly demonstrated the presence of S-specific proteins in both stigma and style. The S allele-protein relationship was found for the S_2, S_3 and S_6 alleles.

From the serological work of Linskens (1960) it is known that S-specific antigens occur in unpollinated *Petunia* styles. The present data are in agreement with those of Linskens. Unpollinated *N. alata* styles contained S-specific proteins, resembling the situation in the sporophytic type of incompatibility (Nasrallah et al. 1970, 1972; Nishio and Hinata 1977, 1978). These results thus support also the assumption of Ferrari and Wallace (1977) that the regulation for sporophytic and gametophytic control systems is basically the same. However, the observations with *Nicotiana* are at variance with the *Petunia* model of Van der Donk (1975) which hypothesizes that the S-specific polypeptides from the styles are synthesized only after pollination.

Assuming that the S-2 and S-3 band are really the incompatibility proteins involved in the recognition reaction, the present results show that the principle of self-compatibility following bud pollination is not based upon the fact that the S-proteins are absent in developing styles. In *N. alata* the shift from compatibility to incompatibility was accompanied by a gradual increase in S-protein concentration, but not by an abrupt increase as has been reported for *Brassica* (Nasrallah 1974; Nishio and Hinata 1977).

It is possible that the principle of self-compatibility following bud pollination may be due to the fact that the S-proteins, although present, are not effective since one or more components of the reactions leading to inhibition of pollen tube growth (rejection reaction) are still absent. This assumption is supported by the demonstration that the shift from compatibility to incompatibility is accompanied by certain metabolic changes which are reflected by the induction of a specific peroxidase (Bredemeijer 1976).

The differences observed in the staining intensity among the S-specific bands, as reported for the sporophytic system (Nishio and Hinata 1978), might not only reflect the actual differences in the concentration of S-proteins, but also the differences in their affinity for the staining reagent or in their solubility in the extraction medium. Each of these factors might explain the failure to detect the S-7 band. Further, it is also possible that other bands might have interfered with the S-7 band as has been the case for the S-6 band on gels with a pH gradient from 3.5 to 9.5.

The nature of the S-specific proteins is still unknown. The peroxidase- and esterase isoenzyme patterns of *N. alata* styles have been shown to be independent of the S-genotype (Bredemeijer and Blaas 1980; Blaas and Bredemeijer 1978). This means that the S-specific proteins are not identical with these enzymes. In spite of the relatively high number of individuals tested the possibility, however small, that these specific proteins are products of the genetic backgrounds cannot be entirely excluded. On the other hand, the fact that the S-specific proteins occurred not only in the stigma as in *Brassica* (Nishio and Hinata 1978), but also in the style supports that these proteins are involved in the incompatibility reaction. In plants with a gametophytic incompatibility system, generally the whole style may act as recognition site because all parts of the style influence pollen tube growth in the same way after selfing (Straub 1946; Lawson and Dickinson 1975).

Further research including other S-alleles with varying genetic backgrounds, isolation and characterization of the specific proteins and a study of their physiological role are required to establish whether indeed incompatibility proteins are involved.

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