Theor. Appl. Genet. 62, 305–309 (1982)

Mechanism of Peroxidase Isoenzyme Induction in Pollinated *Nicotiana alata* Styles

G. M. M. Bredemeijer
Research Institute ITAL, Wageningen (the Netherlands)

Summary. A comparative study on the induction of peroxidase isoenzymes, specifically number 10 (P-10) in *Nicotiana alata* styles revealed significant differences between the various plants of an inbred progeny. In some plants the ageing-induced increase in P-10 activity was very low, whereas in some others, it was relatively high. Pollination accelerated this increase, independent of the pollen genotype. Fertilization was followed by a considerable increase in the activity of several peroxidase isoenzymes, including P-10 in all the plants.

Two plants that differed greatly with regard to P-10 induction were used in additional experiments in order to ascertain the mechanism involved in the induction of P-10. The increase in P-10 activity due to pollination or fertilization can partly be explained on the basis of auxin and auxin-induced ethylene activity. The differences in P-10 induction between various plants of the inbred progeny were probably due to differences in their sensitivity to ethylene.

Key words: Peroxidase isoenzymes – *Nicotiana alata* inbred progeny – Styles – Pollination – IAA treatment

Introduction

Ageing, pollination and fertilization of *N. alata* flowers cause an increase in the activity of several peroxidase isoenzymes in the style (Bredemeijer 1974). Certain peroxidase isoenzymes are influenced by all these processes whereas others are influenced by only one or two of the processes. The peroxidase isoenzyme 10 (P-10), one of the physiological factors that influences incompatible pollen tube growth as well as the incompatibility reaction, is enhanced by ageing, pollination and fertilization.

In a previous investigation with *N. alata,* some plants of an inbred progeny differed in the pollination-induced increase in P-10 activity (Bredemeijer 1978). The events or the factors following pollination which lead to an increase in the activity of P-10 or other peroxidase isoenzymes are still unknown. For this reason a study was undertaken with the aim of comparing P-10 induction in various plants of the inbred progeny with respect to several processes, namely ageing, pollination and fertilization. It is known that these processes cause an increase in the amount of free IAA (Muir 1942, 1947; Lund 1956) and ethylene in the style (Nichols 1977; Bredemeijer 1982), the two growth hormones which are known to induce specific peroxidases in plant tissues (Ritzert and Turin 1970; Lee 1971; Morgan and Fowler 1972; Birecka *et al.* 1976). Therefore, attempts have been made to induce P-10 by treating pistils with IAA and ethrel.

Materials and Methods

The self-incompatible clone OWL (S_2S_3) of *Nicotiana alata* Link and Otto and the inbred progeny used in the present study were the same as described previously (Bredemeijer and Blaas 1980, 1981). The flowers collected at anthesis were emasculated and pollinated. Subsequently they were incubated at 15 °C (8,000 lux, 16 h; darkness 8 h). In some experiments the unpollinated pistils were incubated in 0.01 M phosphate buffer (pH 6.0 or 7.0) with or without IAA (0.05–0.5–5.0 mM) or in 0.05 M phosphate buffer (pH 6.0) containing 0.05 to 0.4% ethrel for 7 days at 15 °C. Ethrel is converted to ethylene within the plant (Henry and Jordan 1977).

The style extracts were prepared by homogenizing 7 styles with 0.5 ml 4% NaCl solution in distilled water in an ice-cooled mortar. The supernatants that were obtained after centrifugation for 45 min at 18,000 g, were used for starch gel electrophoresis of the peroxidase isoenzymes (Bredemeijer 1974).

Results

1 Induction of Peroxidase Isoenzyme P-10 in Ageing Flowers

In plants of clone OWL and its inbred progeny the styles at anthesis contained an extremely low amount of

0040-5752/82/0062/0305/$ 1.00

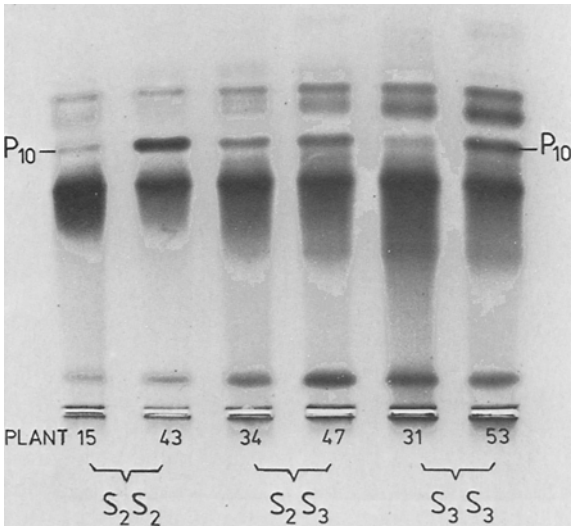

Fig. 1. Stylar peroxidase isoenzyme patterns of aged flowers from different plants of an inbred progeny of the *N. alata* clone OWL

P-10 (Bredemeijer and Blaas 1980). The ageing of these unpollinated flowers caused a gradual increase in the activity of P-10. A comparison of the peroxidase isoenzyme patterns among the various plants of the inbred progeny at 7 days after anthesis has revealed striking differences in P-10 activity (Fig. 1), e.g. plant 31 showed hardly any increase while plant 43 had much higher P-10 activity.

Further, the induction rate of P-10 in plant 31 also remained low during prolonged ageing; after 21 days, when styles already started browning, the P-10 activity was much lower than that found after 7 days in plant 43.

2 Induction of Peroxidase Isoenzyme P-10 During the Progame Phase

The pollination-induced increase in P-10 activity in the inbred progeny also varied from plant to plant. However, the increase in P-10 activity was higher than that of the ageing-induced increase. Further, the rate of increase in the P-10 activity appeared to be similar after self-, or cross-pollination.

The analysis of plant 31 (designated here as "weak-induction type") shows that after incompatible pollination the P-10 activity in the style increased only slightly, irrespective the origin of the pollen (Fig. 2); even after 7 days P-10 activity was still very low. Following a compatible pollination the increase in P-10 activity was also very small until the pollen tubes reached the ovary. This cannot be ascribed to an incapacity of the pollen to induce P-10 because the same pollen induced P-10 in certain other plants (Fig. 3; Bredemeijer 1978). The pollen of plant 31 induced a high P-10 activity in the plants in which ageing induced a relative high P-10 activity and a low P-10 activity in those where the ageing-induced activity was also low.

The pollination of plant 43 (designated here as "strong-induction type") always resulted in a strong increase of P-10 activity in the style independent of the pollen source and the incompatibility reaction (Fig. 3). However, P-10 induction by the pollen of plant 43 after pollination of other plants yielded similar results as were obtained for pollen of plant 31.

3 Induction of Peroxidase Isoenzyme P-10 After Fertilization

In the original clone OWL used in previous studies, compatible pollination caused a considerable increase in the P-10 activity which continued after fertilization

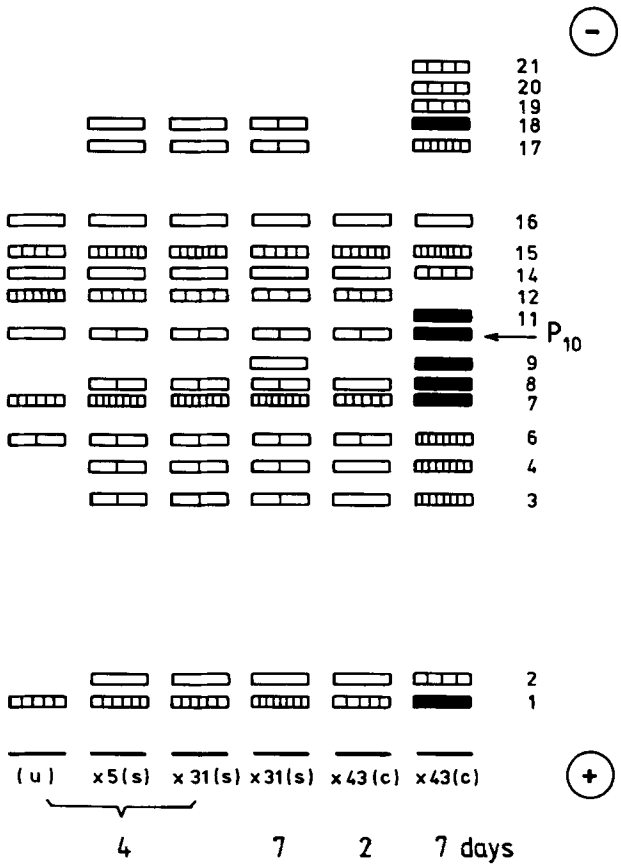

Fig. 2. Peroxidase isoenzyme patterns of the styles from unpollinated (u), self-pollinated (s), and cross-pollinated (c) flowers of the *N. alata* plant 31

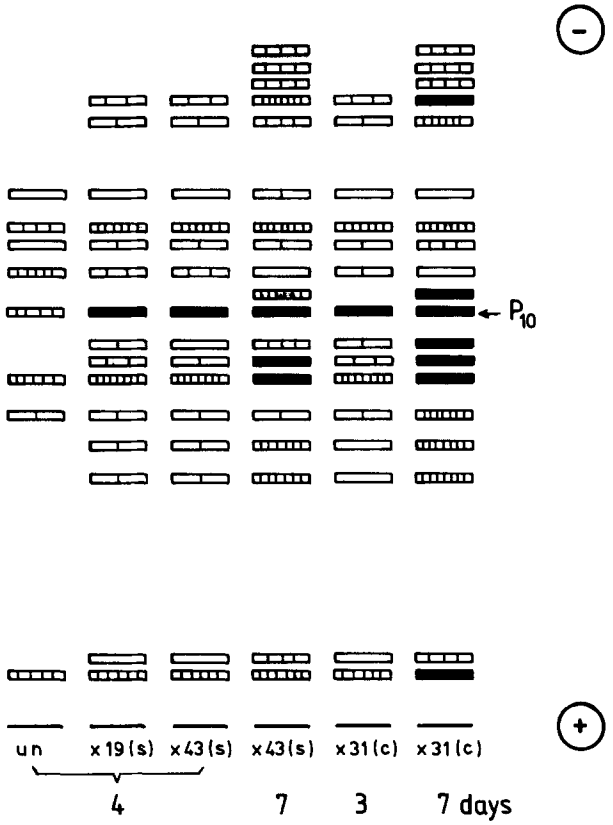

Fig. 3. Peroxidase isoenzyme patterns of the styles from unpollinated (u), self-pollinated (s), and cross-pollinated (c) flowers of the *N. alata* plant 43

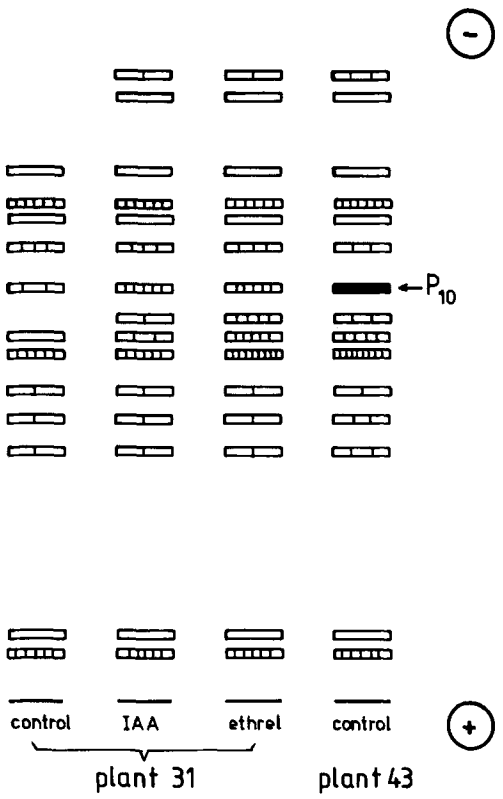

Fig. 4. Effect of IAA (5 mM) and ethrel (0.4%) treatment of pistils on the peroxidase isoenzyme pattern of the styles of plant 31

(Bredemeijer 1974). Since pollen tube growth and fertilization quickly follows one after another, it has not been possible to establish separately the effect of fertilization. However, the availability of plants of the "weak-induction type" (plant 31) in which pollen tube growth induced hardly any P-10 enabled the analysis on the effect of fertilization.

A comparison of the peroxidase isoenzyme patterns in the styles of plant 31 at various times after compatible pollination revealed a strong increase in the P-10 activity starting 3 days after pollination when the first pollen tubes have reached the ovary (Fig. 2). After 7 days, the P-10 activity was approximately similar to that in the plants of "strong induction type" (Fig. 3).

In addition to P-10, several other peroxidase isoenzymes increased in activity after fertilization (Fig. 2). In plant 31, the isoenzymes 11, 19, 20 and 21 were induced only after fertilization.

4 Effects of IAA and Ethrel on Peroxidase Isoenzyme P-10

Ethrel or IAA treatment of unpollinated pistils of plant 31 caused an increase in the activity of certain peroxidase isoenzymes (Fig. 4). The pathway of IAA action on peroxidase activity *via* ethylene might explain the identical effects of IAA and ethrel. The ethylene production after treatment of pistils with IAA was considerably higher than that in the untreated controls (unpublished results).

The P-10 activity also increased in the treated pistils. However, this increase in plant 31 was significantly smaller than that in the untreated pistils of the "strong induction type", plant 43 (Fig. 4). The concentration of IAA and ethrel was probably suboptimal at the site of P-10 synthesis. Maximum induction rate was observed after treatment at 5 mM IAA or 0.4% ethrel; higher concentrations were not tested.

Discussion

The results obtained in the present study with the inbred progeny of *N. alata* show that the ageing-induced increase in P-10 activity of unpollinated styles depended on the genotype of the plant. Among the inbred progeny, the induction of P-10 ranged from very weak ("weak-induction type") to very strong ("strong-induction type"). The pollination accelerated the in-

crease in P-10 activity independently of the extent of penetration of the pollen tubes into the style (see also Bredemeijer and Blaas 1975) and the genotype of the pollen (Fig. 2). After fertilization, P-10 activity was enhanced strongly in all the plants.

From the present data, and from results from other studies, a mechanism has been proposed showing the probable events leading to the P-10 induction (Fig. 5):

i) After pollination the pollen tubes, during penetration of the style, cause a mechanical injury which is accompanied by a peak in ethylene formation (Bredemeijer 1982). Since exogenous ethylene is able to induce P-10 in the style, the injury-induced ethylene peak probably initiates the increase in P-10 activity after pollination (Fig. 5:I). This increase is small and similar to that observed after a mechanical wounding of the stigma with a razor blade.

ii) During the growth of the pollen tubes through the style several substances, e.g. enzymes, co-enzymes, and activators are released by the pollen tubes (Linskens 1968). Some of these substances convert the bound IAA in the style to free IAA (Muir 1947), or stimulate IAA synthesis (Lund 1956). The IAA probably induces the production of ethylene (Hall and Forsyth 1967), which in turn enhances the activity of several peroxidases, including P-10 (Fig. 5:II). Both the ethylene synthesis (Bredemeijer 1982) and the P-10 activity (Bredemeijer 1974) increase steadily during pollen tube growth through the style. It seems likely that the ethylene evolution is, once started, autocatalytic (Burg and Dijkman 1967). Each cell triggers its neighbour to produce ethylene by gassing it with the hormone. This might explain why P-10 is also induced in the basal half of self-pollinated styles in spite of the fact that this part does not contain pollen tubes.

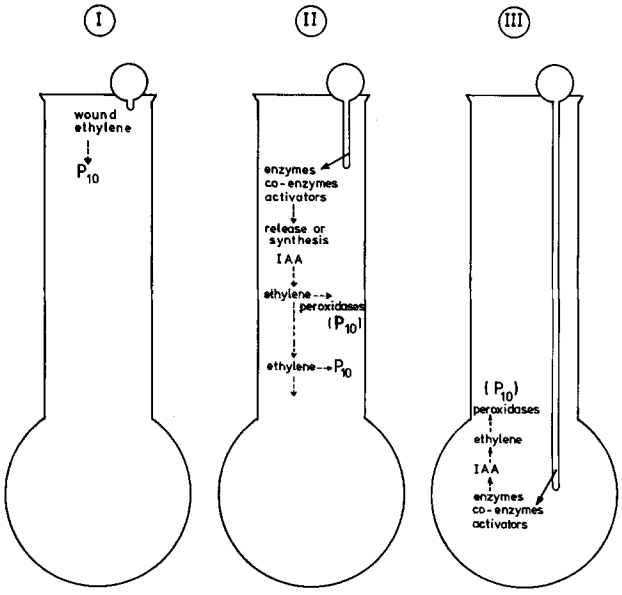

Fig. 5. A scheme showing the probable reactions leading to the induction of peroxidase isoenzyme 10 (P-10) in pollinated *N. alata* styles

It seems likey that the mechanism for P-10 induction in ageing unpollinated styles is essentially the same as that in the pollinated styles. Both auxin (Lund 1956) and ethylene concentration (Bredemeijer 1982) increased during ageing, however, at a much lower rate as compared to the increase in pollinated styles. This is in accordance with the fact that the rate of P-10 induction is also much lower.

The differences in the increase of P-10 activity between plants of the "weak-induction type" and the "strong-induction type" are probably due to their differences in sensitivity to ethylene. While the ethylene production in the two induction types was more or less the same, the ethylene concentration necessary to induce P-10 was significantly higher in plants of the "weak-induction type" (Bredemeijer 1982). The difference in sensitivity of styles to ethylene might be due to differences in the metabolic activity which result in different production rates of CO_2, a competitive inhibitor of ethylene action.

iii) The increase in P-10 activity in the style after fertilization is probably caused by the large amount of IAA which is released (Muir 1942) or synthesized (Lund 1956) in the ovary. The effect of IAA on the P-10 activity and other peroxidases is mediated via ethylene which can diffuse from the ovary to the style (Fig. 5:III). Fertilization not only stimulates the production of free IAA in the ovary, but also of ethylene (Nichols 1977). Moreover, the increase in P-10 activity in IAA-treated pistils of plant 31 was preceded by a surge of ethylene production (Bredemeijer, unpublished results).

In conclusion, it appears that the effects of ageing, pollination and fertilization on P-10 in the style can partly be explained on the basis of auxin and auxin-induced ethylene activity. The amounts of IAA, ethylene and P-10 increase steadily during these processes. Moreover, treatment of the pistils with these growth hormones enhances the P-10 activity.

Acknowledgement

The author wishes to thank Dr. K. Sree Ramulu for critical reading and correction of the manuscript.

Literature

Birecka, H.; Catalfamo, J.; Urban, P. (1976): Cell isoperoxidases in sweet potato plants in relation to mechanical injury and ethylene. Plant Physiol. **57**, 74–79

Bredemeijer, G.M.M. (1974): Peroxidase activity and peroxidase isoenzyme composition in self-pollinated, cross-pollinated and unpollinated styles of *Nicotiana alata.* Acta Bot. Neerl. **23,** 149–157

Bredemeijer, G.M.M. (1978): Variations in the pollination-induced increase in activity of stylar peroxidase isoenzymes in various plants of an inbred progeny of *Nicotiana alata.* Incompatibility Newslett. **9,** 33–42

Bredemeijer, G.M.M. (1982): Ethylene production and peroxidase isoenzymes in *Nicotiana alata* styles. Incompatibility Newslett. **14** (in press)

Bredemeijer, G.M.M.; Blaas, J. (1975): A possible role of a stylar peroxidase gradient in the rejection of incompatible growing pollen tubes. Acta Bot. Neerl. **24,** 37–48

Bredemeijer, G.M.M.; Blaas, J. (1980): Do S allele-specific peroxidase isoenzymes exist in self-incompatible *Nicotiana alata?* Theor. Appl. Genet. **57,** 119–123

Bredemeijer, G.M.M.; Blaas, J. (1981): S-specific proteins in styles of self-incompatible *Nicotiana alata.* Theor. Appl. Genet. **59,** 185–190

Burg, S.P.; Dijkman, M.J. (1967): Ethylene and auxin participation in pollen induced fading of Vanda orchid blossoms. Plant Physiol. **42,** 1648–1650

Hall, I.V.; Forsyth, F.R. (1967): Production of ethylene by flowers following pollination and treatments with water and auxin. Can. J. Bot. **45,** 1163–1166

Henry, E.W.; Jordan, W. (1977): The enzymic response of pea (*Pisum sativum*) stem sections to applied indoleacetic acid, gibberellic acid and ethrel: catalase, peroxidase and polyphenol oxidase. Z. Pflanz. Physiol. **84,** 321–327

Lee, T.T. (1971): Promotion of indoleacetic acid oxidase isoenzymes in tobacco callus cultures by indoleacetic acid. Plant Physiol. **48,** 56–59

Linskens, H.F. (1968): Egg-sperm interactions in higher plants. Accad. Naz Dei Lincei Guaderno N. **104,** 47–56

Lund, H.A. (1956): Growth hormones in the styles and ovaries of tobacco responsible for fruit development. Am. J. Bot. **43,** 562–568

Morgan, P.W.; Fowler, J.L. (1972): Ethylene: modification of peroxidase activity and isozyme complement in cotton (*Gossypium hirsutum* L.) Plant Cell Physiol. **13,** 727–736

Muir, R.M. (1942): Growth hormones as related to the setting and development of fruit in *Nicotiana tabacum.* Am. J. Bot. **29,** 716–720

Muir, R.M. (1947): The relationship of growth hormones and fruit development. Proc. Natl. Acad. Sci. (USA) **33,** 303–312

Nichols, R. (1977): Sites of ethylene production in the pollinated and unpollinated senescing carnation (*Dianthus caryophyllus*) inflorescence. Planta **135,** 155–159

Ritzert, R.W.; Turin, B.A. (1970): Formation of peroxidases in response to indole-3-acetic acid in cultured tobacco cells. Phytochemistry **9,** 1701–1705

Received April 24, 1982
Communicated by H. F. Linskens

Dr. G. M. M. Bredemeijer
Research Institute ITAL
P.O. Box 48
6700 AA Wageningen (the Netherlands)