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THE DISTRIBUTION OF PEROXIDASE ISOENZYMES, CHLOROGENIC ACID OXIDASE AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN TRANSMITTING TISSUE AND CORTEX OF NICOTIANA ALATA STYLES

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SUMMARY

A comparison was made of the activities of peroxidase, chlorogenic acid oxidase, glucose-6-phosphate dehydrogenase and of peroxidase isoenzymes in the transmitting tissue and the cortex of unpollinated *Nicotiana alata* styles.

Whereas most peroxidase and chlorogenic acid oxidase activity in a style occurred in the cortex, the activity of glucose-6-phosphate dehydrogenase, on the contrary, was highest in the extracts of transmitting tissue.

Comparison of peroxidase and glucose-6- phosphate dehydrogenase activities in the style extracts with the sum of the activities present in the cortex and transmitting tissue, when measured separately revealed discrepancies which can be explained by the presence of enzyme inhibitors.

Starch gel electrophoresis showed that two peroxidase isoenzymes occur in the transmitting tissue, namely No. 12, a major one which was specific for this tissue and a minor one, No. 7 that was also found in the cortex. The peroxidase isoenzyme No. 10, which has previously been suggested to be involved in the regulation of pollen tube growth occurs only in the cortex, thus indicating that it influences the pollen tube growth indirectly.

The possible interactions between chlorogenic acid and the enzymes, peroxidase and chlorogenic acid oxidase in relation to pollen tube growth are discussed.

1. INTRODUCTION

In previous studies on peroxidase activity and its possible influence on pollen tube growth in *Nicotiana alata* styles (BREDEMEIJER 1974, 1976 and 1977; BREDEMEIJER & BLAAS 1975) no data have been reported on a possible tissue-specific distribution of the isoenzymes in the style.

Electrophoretic investigations have indicated that the various plant tissues possess different peroxidases (see SCANDALIOS 1964; MACNICOL 1966; GORDON & ALLDRIDGE 1971; LABERGE 1975). It is suggested that these peroxidases have different substrate (hydrogen donor) requirements and several metabolic functions in the plant. These functions may vary with the tissue or with the subcellular organelle (EVANS & ALLDRIDGE 1965; WISE & MORRISON 1971). Knowledge of the distribution pattern of various peroxidase isoenzymes in the style appears, therefore, basic to an understanding of the role which the peroxidases may play in the regulation of pollen tube growth.

A *Nicotiana alata* style is not a homogeneous tissue, but contains four elements,

namely the epidermis, the cortex together with the vascular tissue system and the transmitting tissue, similar to that shown by BELL & HICKS (1976) for *N. tabacum* styles. Since the transmitting tissue serves as the medium through which the pollen tubes grow en route to the ovules it is important to know which peroxidase isoenzymes occur in this tissue. In a previous study it has been demonstrated that peroxidase was able to inhibit pollen tube growth *in vitro* (BREDEMEIJER 1975).

In the past, dissection of transmitting tissue from styles was successfully carried out by LINSKENS (1955) and KROH (1973). By this method two fractions were obtained: transmitting tissue and cortex, the latter including epidermal tissue, ground parenchyma and vascular traces.

Except peroxidase the localization was studied of two other enzymes, namely glucose-6-phosphate dehydrogenase (G-6-PDH) that was used as a plasma marker enzyme in studies dealing with extracellular stylar peroxidases (BREDEMEIJER 1977) and chlorogenic acid oxidase (CAO) which may regulate the activity of enzymes like peroxidase (KOSUGE 1969).

2. MATERIAL AND METHODS

Flowers of *Nicotiana alata* Link et Otto (clone OWL, S_2S_3) were collected at anthesis. After removal of the stigma the transmitting tissue was dissected under a stereo microscope from the topmost 2 cm of 12 styles. Extraction of peroxidase, CAO and G-6-PDH with a 4% NaCl solution, enzyme assays and starch gel electrophoresis of peroxidase isoenzymes were carried out by the same procedures as described in previous papers (BREDEMEIJER 1973, 1974, 1976, 1977). The activity of CAO was determined by measuring the decrease in absorbance of chlorogenic acid at 326 nm as described by BATRA & KUHN (1975).

For comparison of peroxidase isoenzymes from various tissues and organs of the plant equal amounts of wet weight (0.1 g) were homogenized in an ice-cooled mortar with pure quartz sand and 0.5 ml 4% NaCl solution. Pollen extracts were prepared by homogenizing 50 mg pollen in an ice-cooled Potter Elvehjem homogenizer with 1.1 ml 4% NaCl solution.

3. RESULTS

3.1. Activities of peroxidase, CAO and G-6-PDH in the transmitting tissue and cortex of styles

The mean activities of peroxidase, CAO and G-6-PDH in extracts from cortex, transmitting tissue and style are summarized in *table 1*. The activities of peroxidase and CAO in the cortex were respectively three and ten times more than those present in the transmitting tissue. The activity of G-6-PDH, on the contrary, was low in the cortex and relatively high in the transmitting tissue.

A comparison between the enzyme activity in style extracts and the sum of the activities of cortex and transmitting tissue, when measured separately, revealed lower peroxidase and G-6-PDH activities in the stylar extracts as expected (*table 1*); the CAO activity in stylar extracts was the sum of the parts.

Table 1. Mean activities of chlorogenic acid oxidase, peroxidase and glucose-6-phosphate dehydrogenase in different tissues of *Nicotiana alata* styles. Data are the average of three experiments.

	CAO		Peroxidase		G-6-PDH	
	ΔA326/style/5 min	%	ΔA334/style/5 min	%	ΔA340/style/5 min	%
Cortex	2.076 ± 0.424	91	0.090 ± 0.019	73	0.004 ± 0.001	13
Transmitting tissue	0.202 ± 0.019	9	0.034 ± 0.006	27	0.026 ± 0.003	87
Style (found)	2.458 ± 0.615		0.067 ± 0.018		0.008 ± 0.002	
Style (calculated)	2.278 ± 0.429		0.124 ± 0.023		0.030 ± 0.003	

3.2. Peroxidase isoenzymes in the transmitting tissue and cortex of styles

Figure 1 shows an electrophoretic separation of the peroxidase isoenzymes from the styles collected at anthesis and from the styles aged during 7 days at 15 °C. The latter were used to study the localization of peroxidase isoenzyme No. 10 which was present only in aged or pollinated styles.

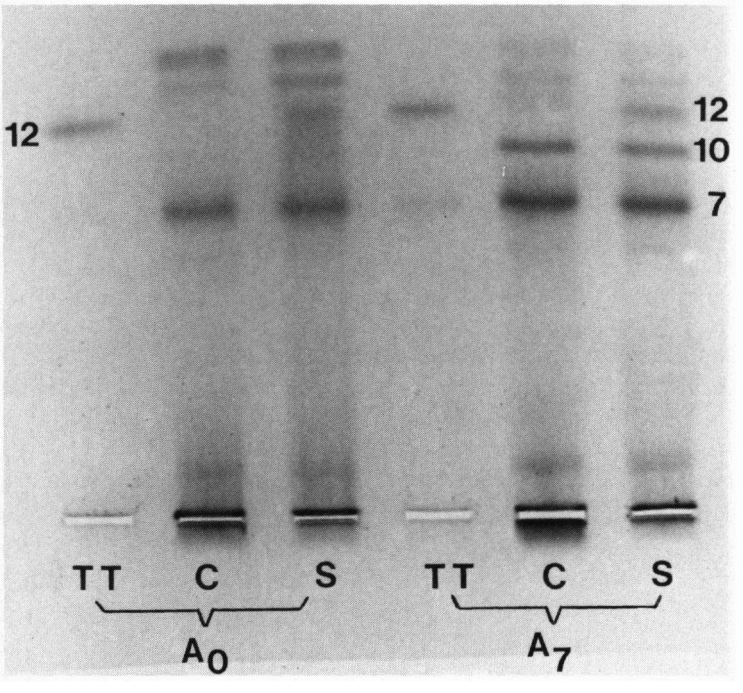

Fig. 1. Electrophoretic separation of the peroxidase isoenzymes of the transmitting tissue (TT), cortex (C) and undissected tissue (S) from *Nicotiana alata* styles collected at anthesis (A_0) and from the aged styles (A_7).

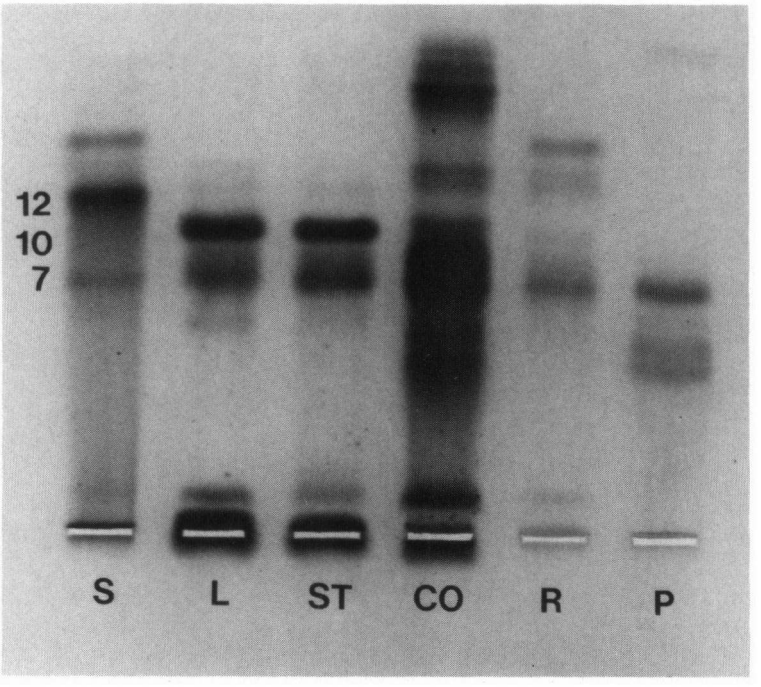

Fig. 2. Electrophoretic separation of the peroxidase isoenzymes located in *Nicotiana alata* tissues and organs. The style extract was concentrated twice. S, style at anthesis; L, leaf; ST, stem; CO, corolla; R, root; P, pollen.

The transmitting tissue contained two peroxidase isoenzymes, namely No. 12, a major one which is specific for this tissue and a minor one, No. 7, that also occurs in the cortex. All the other stylar peroxidase isoenzymes including No. 10 which has previously been suggested to be involved in regulation of pollen tube growth occurred only in the cortex. The comparison of peroxidase isoenzyme patterns of the various tissues and organs of a *N. alata* plant revealed that peroxidase No. 12 occurred only in the style (*fig. 2*); No. 7 was the only one which was common to all the plant parts.

In contrast with what one should expect from the results in *table 1* no differences could be observed between the intensities of the isoenzymic bands of the style and the intensities of the corresponding bands in the patterns of cortex and transmitting tissue (*fig. 1*). It is likely that an inhibitor present in the style extracts is lost by the electrophoretic procedure.

4. DISCUSSION

4.1. Peroxidase

The fact that the transmitting tissue is the only part of *N. alata* plants which contains the peroxidase isoenzyme No. 12 (*fig. 2*) suggests at least that the role of

this isoenzyme is specifically correlated with the physiological function of the style. Preliminary experiments indicate that irradiation-induced inactivation of peroxidase 12 is accompanied by a reduction of compatible pollen tube growth. Since irradiation causes several biochemical changes it would, however, be premature to ascribe the observed reduction of pollen tube growth to an inactivation of peroxidase isoenzyme No. 12.

In contrast to peroxidase isoenzyme No. 12, isoenzyme No. 10, which has previously been suggested to be involved in the regulation of pollen tube growth (BREDEMEIJER & BLAAS 1975; BREDEMEIJER 1977 and 1978) occurs only in the cortex. Since the extracts of the transmitting tissue never contained even a trace amount of peroxidase No. 10, in spite of the fact that a rather crude dissection method was used, it is suggested that this isoenzyme is not localized in tissue immediately surrounding the transmitting tissue, but possibly in the vascular tissues which are known to contain much peroxidase (VAN FLEET 1952; MACNICOL 1966; GORDON & ALLDRIDGE 1971; SHEEN 1973). Therefore, peroxidase No. 10 should influence pollen tube growth indirectly, probably *via* reaction products which might migrate from cortex to transmitting tissue. The fact that peroxidase No. 10 occurs not only in the style, but also in leaves and stems (*fig. 2*) indicates that the physiological role of this isoenzyme is certainly not specifically correlated with regulation of pollen tube growth. In a previous study it has already been established that peroxidase No. 10 is not necessary for growth inhibition of incompatible pollen tubes but that it is one of the factors which might influence tube growth besides the incompatibility reaction itself (BREDEMEIJER 1978).

The peroxidase activity in the style extracts is approximately half the sum of the activity of cortex and transmitting tissue extracts when measured separately (*table 1*). Probably, one or more peroxidase isoenzymes are inhibited by substances released or produced during homogenization of the tissue. Possibly chlorogenic acid is involved in this inhibition. SHEEN (1969) suggested that the low peroxidase activity in *N. tabacum* flower parts may be caused by the high chlorogenic acid content.

4.2. Glucose-6-phosphate dehydrogenase

The low G-6-PDH activity in the cortex and style extracts on the one hand and the relatively high activity in transmitting tissue extracts (*table 1*) on the other indicate the presence of an inhibitor in the cortex. In the style extracts this inhibitor comes into contact with the G-6-PDH originating from the transmitting tissue.

As the cortex has a high concentration of chlorogenic acid, an effective inhibitor of G-6-PDH (FIRENZUOLI et al. 1969), it is possible that this phenolic compound is the enzyme inhibitor in style extracts. The absence or very low concentration of chlorogenic acid in the transmitting tissue (unpublished data) then explains the relatively high G-6-PDH activity in the extracts of this tissue.

4.3. Regulation of enzyme activity and pollen tube growth in the style by the CAO-chlorogenic acid system

It is not known at the moment whether the spatial separation of certain enzymes

and phenolic inhibitors in the style has a physiological significance. SHEEN (1969) expressed the view that the polyphenols in the pistil may perform roles such as regulating enzymic activity essential for flower development and/or sexual reproduction.

It is possible that the increased permeability of cell membranes after pollination (BREDEMEIJER 1977) enables release of chlorogenic acid from the cortex to the transmitting tissue. Chlorogenic acid has been reported to migrate in plants (SHEEN 1969). We have no data as yet to ascertain whether chlorogenic acid inhibits *N. alata* pollen tube growth; it is known in *Crinum* that the pollen tube growth *in vitro* is not influenced (MARTIN 1972). However, the situation *in vivo* is quite different. Chlorogenic acid can inhibit enzymes like peroxidase and G-6-PDH, which in turn may influence pollen tube growth. Furthermore, chlorogenic acid can be oxidized to quinones by polyphenol oxidases (e.g. CAO) and peroxidases (e.g. peroxidase isoenzyme No. 12; unpublished data) present in the style. The interference of quinones with several metabolic processes may cause inhibition of pollen tube growth similarly as reported for the antimicrobial activity of these substances (KOSUGE 1969).

Because of the high CAO activity in the cortex it is also possible that chlorogenic acid is already oxidized in this tissue. The question is then whether the oxidation products can still migrate to the transmitting tissue or whether they are polymerized, forming melanins, which leads to browning of the tissue. As the latter process takes place only after pollen tube growth has been completed it is not important anymore for regulation of pollen tube growth.

The possible interactions are numerous and the interplay of various phenolic compounds and enzymes involved in their metabolism deserves further attention in studies of style-pollen tube interaction.

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