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ARABINOGALACTANS ARE COMMON COMPONENTS OF ANGIOSPERM STYLES

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Key Word Index—Angiosperms; arabinogalactans; arabinogalactan-proteins; style components; Yariv antigens.

Abstract—Stylar extracts from flowers of 18 angiosperms were screened by means of artificial carbohydrate antigens, and arabino-3,6-galactans were found to be present in all cases.

INTRODUCTION

Components of the female sexual tissues of angiosperms which participate in the recognition and adhesion of compatible pollen, as well as the nourishment of pollen tubes growing through the style towards the ovary, are incompletely defined. However, a role for arabino-3,6-galactans in adhesion of pollen and nourishment of pollen tubes has been proposed (for review, see ref. [1]). Arabinogalactans associated with protein have been isolated from styles of three flowering plants, *Gladiolus gandavensis* [2, 3], *Lilium longiflorum* [4] and *Nicotiana alata* [5], and in each case account for a high proportion of the total soluble carbohydrate in the extracts. In this study we have screened style extracts prepared from a range of different plants and have shown that arabino-3,6-galactans are common style components.

RESULTS AND DISCUSSION

We screened the style extracts using artificial carbohydrate antigens. These are a class of dyes prepared by coupling diazotized *p*-aminophenyl glycosides to phloroglucinol and were first used by Yariv *et al.* to precipitate arabinogalactans from plant extracts [6]. They have been used extensively both as precipitants and cytochemical reagents to detect arabinogalactans and arabinogalactan-proteins* in tissue extracts and sections [7, 8]. The exact nature of the interaction is not known; however, the glycosyl residues of the artificial antigen must be in the β-configuration for them to interact with arabinogalactans [7–11]. In this study we tested the style extracts against the water-soluble β-glucosyl artificial carbohydrate antigen in a double-diffusion test; a red precipitin band is formed when arabinogalactan is present [12]. In control experiments the α-galactosyl artificial carbohydrate antigen was used. In principle, any of the α-glycosyl artificial carbohydrate antigens could be used in control experiments, as it is the anomeric configuration which is the key feature of the dye in determining its property of precipitating arabinogalactans. In practice, the α-glucosyl artificial carbohydrate antigen is poorly soluble in aqueous solutions and the more soluble α-galactosyl analogue was used. An estimate of the minimum amount of arabinogalactan in the different style extracts was calculated from the fresh weight of styles used to prepare the extract and from the finding that 15 μg/ml is the minimum concentration of defined arabinogalactan (from *Gladiolus* styles [1, 2]) which can be detected by this method. This was done by serial two-fold dilution of a stock solution of isolated arabinogalactan (1 mg/ml) and double diffusion of the diluted solution against the β-glucosyl artificial carbohydrate antigen; solutions of concentration of 15 μg/ml or more gave a single band, but no band was detected when solutions of 7.5 μg/ml or less were used. The figures quoted in Table 1 are only a guide to the relative amounts of arabinogalactans present; it is possible that individual arabinogalactans with different minor components may be differentially sensitive to precipitation with the Yariv artificial carbohydrate antigen.

We are restricted in this study to flowers with accessible and relatively large pistils; plants such as *Acacia* species (Mimosaceae) and *Pisum sativum* (Fabaceae) have such small styles that it is not practicable to collect sufficient material to prepare extracts at the concentrations required. The results (Table 1) indicate that arabino-3,6-galactans are common components of style extracts of both monocotyledons and dicotyledons. It is possible that these arabinogalactans, like those from other sources, are covalently associated with protein, but an analysis of the isolated arabinogalactans would be required to demonstrate this.

Arabinogalactans and arabinogalactan-proteins are widely distributed in plant tissues and exudates, but no function for this class of molecules has yet been established although they have been implicated in a range of biological phenomena [7, 8]. The possibility that style arabinogalactan-proteins play a role in pollination is speculative. It is given support, but is not proven, by the following observations:

(1) They are major style components in *Gladiolus* [1, 2], *Lilium* [3, 4] and *Nicotiana alata* [5].

*In some cases, protein is covalently associated with arabinogalactans; in other cases, protein may be isolated with the arabinogalactan but the nature of the linkage is not established [7, 8].

Table 1. Presence of arabinogalactans in extracts of styles

Plant families and species from which style extract was prepared*	No. of precipitin bands formed with β-glycosyl artificial carbohydrate antigen†	Estimated amount of arabinogalactan per g fr. wt of material (mg/g)‡
DICOTYLEDONS		
Magnoliaceae		
Magnolia soulangiana	1 (sharp)	0.03
Rosaceae		
Prunus avium	1 (sharp)	1.40
Fabaceae		
Trifolium repens	1 (sharp)	6.25
Vicia faba	1 (sharp)	1.02
Lupinus albus	1 (sharp)	2.00
Proteaceae		
Grevillea sp.	1 (sharp)	0.01
Myrtaceae		
Callistemon sp.	1 (sharp)	0.03
Leptospermum scoparium cv Lambethi	1 (sharp)	4.50
Onagraceae		
Fuchsia cv	1 (sharp)	2.50
Solanaceae		
Nicotiana tabacum	2 (sharp)	1.12
Nicotiana alata	1 (diffuse)	0.03
Lycoperiscon esculentum	1 (sharp)	0.20
MONOCOTYLEDONS		
Cymodoceaceae		
Amphibolis antarctica	1 (sharp)	0.63
Poaceae		
Lolium perenne	1 (diffuse)	0.02
Liliaceae		
Lilium longiflorum	2 (sharp)	N.D.
Narcissus pseudo-narcissus	1 (sharp)	0.70
Iridaceae		
Gladiolus gandavensis	2 (sharp)	2.00
Xanthorrhoeaceae		
Xanthorrhoea australis	2 (sharp)	3.30

*Classified according to Cronquist [13].

†The presence of arabinogalactans in style extracts is shown by the formation of precipitin bands during double-diffusion tests of the extract against the β-glucosyl artificial carbohydrate antigen. The number of bands formed and their appearance give some indication of the nature of the arabinogalactans.

‡This figure was calculated from the concentration of the style extract (fr. wt/ml) and the minimum concentration of arabinogalactan required to give a precipitin band (15 μg/ml).

N.D., Not determined.

(2) They are localized in the tissue through which pollen tubes grow. *Gladiolus* has a hollow style filled with mucilage which contains arabinogalactan as a major component; pollen tubes grow through this mucilage-filled transmitting tract [1]. *Nicotiana alata* has a solid transmitting tissue through which pollen tubes grow extracellularly; the arabinogalactan is localized in the extracellular cell-wall material of the transmitting tract [5].
(3) They are developmentally regulated in *Gladiolus* and *N. alata*; that is, they appear in the tissues of the style at the same time that the style becomes receptive to pollen ([2]; Hoggart, R. M. and Gell, A., unpublished observations).
(4) There is variation in the fine structure of the arabinogalactans isolated from different plants [7, 8].

The present study allows us to add a further point:

(5) They are common components of styles of angiosperms.

EXPERIMENTAL

Flowers used in this study were collected from plants grown locally. The β-glucosyl and α-galactosyl artificial antigens were a

gift from Dr. M. A. Jermyn (Division of Protein Chemistry, CSIRO, Parkville, Victoria, Australia) and were prepared by coupling diazotized 4-aminophenyl glycosides with phloroglucinol [12].

Preparation of crude extracts. Mature styles were collected and stored at −20°. Extracts were prepared by grinding frozen styles (0.15 g fr. wt per 10 ml buffer) in 0.05 M Tris–HCl, 0.15 M $CaCl_2$, pH 7.4 at 4° in a mortar and pestle. The extracts were centrifuged at 12000 *g* for 20 min at 4°; the supernatants were removed, dialysed exhaustively against distilled water, and freeze-dried.

Gel diffusion. Double diffusion was carried out using the microslide method (Gelman Instrument Co., Ann Arbor, MI, U.S.A.) in 1% (w/v) agarose (Calbiochem, San Diego, CA, U.S.A.) containing 0.15 M NaCl and 0.2% NaN_3, for 20 hr at room temp. in a humidity chamber. The β-glucosyl and α-galactosyl artificial antigens were used at 2 mg/ml in 0.15 M NaCl. The freeze-dried style extracts were used at 10 mg/ml (w/v) in 0.005 M NaPi buffer, 0.15 M NaCl, pH 7.4 (PBS). The slides were examined for the presence of red precipitin bands [1]. The lowest concn of arabinogalactan isolated from *Gladiolus* styles which gave a detectable band was 15 μg/ml.

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