Localisation and expression of arabinogalactan-proteins in the ovaries of *Nicotiana alata* Link and Otto

**Abstract** The transmitting-tissue cells of the style of flowering plants secrete a complex extracellular matrix through which pollen tubes grow to the ovary to effect fertilisation. This matrix is particularly rich in a class of proteoglycans, the arabinogalactan-proteins (AGPs). AGPs from the ovary of *Nicotiana alata* were found to be developmentally regulated, as the different charge classes of AGPs altered during floral development. The AGPs from the mature ovary had charge characteristics that were distinct from those previously reported for the stigma and style. However, the concentration of AGP (0.6 µg/ml fresh weight) in the ovary did not change during development, or in response to either compatible or incompatible pollination. The AGPs of the ovary are mainly associated with the epidermis of the placenta.

**Key words** Arabinogalactan-proteins • Ovary • *Nicotiana* • Pollination • Flower development

**Introduction**

In flowering plants the pistil provides an environment suitable for the germination of pollen and for the growth of pollen tubes through the transmitting tissue to the ovules where fertilisation occurs (Knox 1984). The pathway of pollen tube growth is through an extracellular mucilage which provides a complex growth medium for the pollen tubes. In addition to low-molecular-weight metabolites such as sucrose and amino acids, the mucilage contains a range of glycoproteins including the self-incompatibility ribonucleases (S-RNases; Anderson et al. 1986) and several proline-rich proteins (Chen et al. 1992), a 120-kDa glycoprotein (Lind et al. 1994) and arabinogalactan-proteins (AGPs; Hoggart and Clarke 1984). AGPs have been detected at high concentrations in pistil extracts of many species of flowering plants (Hoggart and Clarke 1984). They may be involved in the adhesion of pollen to the stigma surface, may provide a nutrient supply to growing pollen tubes, may play a role in control of water balance, or could act as antimicrobial or anti-fungal agents (Labarca and Loewus 1972, 1973; Fincher et al. 1983).

AGPs of stigmas and styles of *Nicotiana alata* are developmentally regulated. The amount of AGP in these organs increases during floral development from green bud to mature flower, as shown both by direct quantitation of buffer-soluble AGPs using the β-glucosyl Variv reagent (Gell et al. 1986) and by quantitative immunocytochemistry using a monoclonal antibody directed to terminal α-L-arabinofuranosyl residues (Sedgley and Clarke 1986). Following pollination with either compatible or incompatible pollen, the amount of AGP increases in the stigma, but not in the style, of *N. alata* (Gell et al. 1986). However, neither of these studies investigated changes in expression of AGPs in the ovary. This paper describes the changes that occur in the AGPs in the ovary of *N. alata* during flower development and following pollination.

**Materials and methods**

**Plant material**

Seeds of *Nicotiana alata* Link and Otto plants (self-incompatibility genotypes $S_2S_2$ and $S_3S_3$) from Dr K.K. Pandey (DSIR, Palmerston North, New Zealand) were grown in pots in a glasshouse as previously described (Gell et al. 1986). *Lycopersicon peruvianum* (L.) Mill. plants were also grown in pots in a glasshouse, and pollen was collected from mature flowers.

All *N. alata* flowers were emasculated at the beginning of petal colouration. Flowers were collected at seven different stages of development: green bud, elongated green bud, petal formation, petal coloration, maturity (indicated by the presence of a sticky exudate on the stigma surface), 24 h post-maturity and 48 h post-maturity. In addition, some flowers were hand-pollinated at maturity and collected 48 h later. Mature stigmas (genotype $S_2S_3$) were pollinated using either compatible pollen from plants of genotype $S_2S_2$ or
incompatible pollen from plants of genotype $S_S$. In control experiments, pistils were either not pollinated, or were pollinated with non-viable pollen (compatible and incompatible) or with synthetic polyacrylamide beads (Biogel P2). Non-viable pollen was prepared by treating pollen with ethanol vapour for 3 h. In each treatment eight flowers were pollinated. One of the flowers was examined microscopically for pollen-tube growth by squashing the pistil and staining with decolorised aniline blue (see below). The remaining seven pistils were dissected from the flowers and AGPs were extracted as described below.

Chemicals

The \(\beta\)-glucosyl Yariv and \(\alpha\)-galactosyl Yariv reagents were from Biosupplies Australia (Parkville, Victoria, Australia). Gum arabic and bromophenol blue were purchased from Sigma. Agarose was from Calbiochem. Aniline blue was from Merck. All other chemicals were analytical reagent grade unless otherwise specified.

Histological procedures

Longitudinal sections from ovaries of fresh \textit{N. alata} flowers were cut by hand with a razor blade and used directly in cytochemical tests. Sections were stained for 20 min using \(\beta\)-glucosyl Yariv reagent (2 mg/ml in 1% w/v NaCl) as a bright-field colour reagent, and then washed twice in 1% w/v NaCl. As a control for the staining specificity, sections were treated in the same way with \(\alpha\)-galactosyl Yariv reagent, which does not react with AGPs (Jermyn 1978). Sections were viewed with a Wild Photomakroskop M400 and an Intralux 150H lighting unit. The extent of pollen-tube...