ACTA BIOLOGICA CRACOVIENSIA Series Botanica 47/1: 139–146, 2005

IMMUNODETECTION OF ARABINOGALACTAN PROTEINS IN DIFFERENT TYPES OF PLANT OVULES

BARBARA CHUDZIK, BARBARA ZARZYKA, AND RENATA ŚNIEŻKO*

Department of Cell Biology, Maria Curie-Skłodowska University, ul. Akademicka 19, 20-033 Lublin, Poland

Received December 1, 2004; revision accepted March 10, 2005

Arabinogalactan proteins (AGPs) are a diverse class of highly glycosylated plant cell surface proteoglycans. They are discussed as signal molecules participating in cell-cell interaction or cellular signalling during morphogenetic processes. AGPs are abundant in the stigma and transmitting tissue of different flowering plants, and are supposed to play an important role in pollen tube adhesion and guidance. In the present work we localized two epitopes of AGPs using the monoclonal antibody JIM 8 and JIM 13 in ovules of anatomically different types. Although some differences in the localization of these epitopes in different plant species were observed, in all the studied ovules they were present on the pathway of pollen tube growth. In particular plant species they appeared at the time of the ovule's highest receptivity.

Key words: *Galanthus nivalis*, *Galtonia candicans*, *Oenothera*, *Sinapis alba*, arabinogalactan proteins (AGPs), JIM 8 Mab, JIM 13 Mab, ovules.

INTRODUCTION

Arabinogalactan proteins (AGPs) are a diverse class of highly glycosylated plant cell surface proteoglycans. The protein backbone, rich in hydroxyproline, serine and threonine, makes up only 1–10% of the mass of the whole molecule. The glycan components consist of β(1→3)-linked galactan backbones with highly varied branches containing arabinose, galactose and other sugars (Fincher et al., 1983; Serpe and Nothnagel, 1999). The expression of epitopes of AGPs on the cell surface is spatially and temporally regulated in plant development, and often directly reflects the cell fate (Knox et al., 1989; Pennell and Roberts, 1990; Knox et al., 1991; Knox, 1995, 1996, 1999). The proteoglycans of this class are discussed as signal molecules participating in cell-cell interaction or cellular signalling during morphogenetic processes (Knox et al., 1991).

AGPs are abundant in generative organs of flowering plants. They are supposed to play an important role in pollen tube adhesion and guidance. Using the specific interaction of AGPs with an artificial dye, Yariv reagent (Yariv, 1967), AGPs have been found on the stigma surface and in the transmitting tissue of the style in *Gladiolus gandavensis*, *Lilium longiflorum*, *Lycopersicon peruvianum* and *Nicotiana alata* (Clarke et al., 1979; Gleeson and Clarke, 1979, 1980; Sedgley et al., 1985; Sedgley and Clarke, 1986, Webb and Williams, 1988) and on the placenta surface in *Nicotiana alata* (Gane et al., 1995a,b).

At the molecular level, a few hydroxyproline-rich glycoproteins from the stigma and the transmitting tissue of *Nicotiana alata* and *Nicotiana tabacum* have been described (Goldman et al., 1992; Cheung et al., 1993; Chen et al., 1993; Lind et al., 1994; Du et al., 1996; Sommer-Knudsen et al., 1996).

In ovules the presence of AGPs has been shown using monoclonal antibodies against sugar moieties in *Brassica napus*, *Amaranthus hypochondriacus* and *Actinidia deliciosa* (Pennell et al., 1991; Coimbra and Salema, 1997; Coimbra and Duarte, 2003).

AGPs are suggested to play a key role in pollen tube growth as attractants and adhesion molecules, as a source of nutrients and building material, and as a substance that forms a hydrated environment (Cheung et al., 1995; Wu et al., 1995).

In the present study, the pattern of distribution of two AGP epitopes (recognized by JIM 8 and JIM 13 Mab) was investigated in anatomically different types of ovules.

* e-mail: cellbiol@biotop.umcs.lublin.pl

PL ISSN 0001-5296

MATERIALS AND METHODS

PLANT MATERIAL

Model plants were chosen to represent anatomically different types of ovules and to compare fertile ovules with sterile ones. We investigated the following plants: *Galtonia candicans* L. (Liliaceae) and *Galanthus nivalis* L. (Amaryllidaceae), with tenuinucellar ovules (about 40% of *G. nivalis* ovules do not contain an embryo sac and are sterile); *Oenothera hookeri* L. (Oenotheraceae) and *Oenothera* mut. *brevistylis* (female sterile form), with crassinucellar ovules; and *Sinapis alba* L. (Brassicaceae), with ovules having a degraded micropylar nucellus at maturity.

The biology of flowering and the ovule anatomy and morphology of the investigated plants were described in previous papers (Chudzik and Śnieżko, 1999a,b, 2003; Chudzik et al., 2002; Chudzik, 2003).

Flowers of *Galtonia candicans*, *Sinapis alba*, *Oenothera hookeri* and *Oenothera* mut. *brevistylis* were grown in the experimental garden of the Biology Institute of Maria Curie-Skłodowska University in Lublin. Genetically stabile forms of *Oenothera* taxons were maintained every year by selfing. Female sterile plants of *Oe.* mut *brevistylis* were selected according to morphological features from the progeny of a fertile plant. Flowers of *Galanthus nivalis* were received from the Botanical Garden of Maria Curie-Skłodowska University in Lublin. The selected plants were isolated with bags to prevent uncontrolled pollination, and some flowers were hand-pollinated.

The ovules of *G. candicans*, *S. alba*, *Oe. hookeri* and *Oe.* mut. *brevistylis* were taken at three developmental stages: (1) from the bud, at the stage of the 2- or 4-nucleate embryo sac; (2) from the freshly opened, unpollinated flower (embryo sac already fully developed); and (3) a few hours after cross-pollination, after penetration by the pollen tubes into the ovary (timing depended on the species). Due to the specific biology of *G. nivalis* flowering, its ovules were collected in (1) September, at the stage of the fully formed embryo sac; (2) March, from open, unpollinated, isolated flowers, two and five days after opening of the flower; and (3) March, from opened flowers, 48 h after cross-pollination. Sterile ovules were scattered among the fertile ones in the same ovary, enabling comparison of the two types of ovules at the same developmental stage.

OBSERVATIONS OF POLLINATION AND POLLEN TUBE GROWTH

Pollen tube growth and penetration into the ovules was observed with a fluorescence microscope (Nikon, UV 430 nm) after aniline blue staining, as described in previous papers (Chudzik and Śnieżko, 1997). These observations allowed us to estimate the time of the ovules' highest receptivity.

FIXATION AND EMBEDDING

Immediately after isolation, the ovules were fixed in a mixture of 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M phosphate buffered saline (PBS), pH 7.4, placed in a vacuum for 2 h and then kept at 4°C overnight. After fixation, the material was rinsed 3 times in PBS, pH 7.4, and dehydrated in a graded ethanol and acetone series diluted with PBS. Then the samples were embedded in LR-White acrylic resin (medium grade, SIGMA) and left to polymerize for 2 days at 50°C.

IMMUNOLABELLING

For immunofluorescence, 4 μm sections were mounted on POLYSINE^{TM} glass slides (Menzel Glaser, U.S.A.), dried at room temperature and hydrated 2 × 15 min. Sections were preincubated with 4% bovine serum albumin (BSA, Sigma) and 0.05 Tween 20 in 0.01 M PBS, pH 7.4, for 30 min to avoid nonspecific binding of antibodies. Then the slides were washed in PBS for 15 min and incubated with rat monoclonal antibodies (Mab): JIM 13 detecting the trisaccharide β-D-glucose A-β (1→3)-D-galactose A-α (1→2)-L-rhamnose (Knox et al., 1991; Yates et al., 1996) or JIM 8 recognizing sugar chains of AGPs of unknown structure (Pennell et al., 1991; Yates et al., 1996). The monoclonal antibodies were kindly provided by Professor Keith Roberts of the John Innes Centre, Norwich, U.K.

Incubation in medium containing Mab 1:20 (JIM 8, JIM 13) in 0.01 M PBS, pH 7.4, supplemented with 1% BSA and 0.05% Tween 20 was conducted for 24 h at 4°C. After rinsing with PBS, the sections were incubated with secondary FITC-conjugated rabbit anti-rat IgGs (Sigma) diluted 1:50 in the same buffer for 24 h at 4°C in the dark. After labelling, the sections were washed with PBS 2 × 20 min, then washed with distilled water 5 × 15 min, and finally enclosed in Mounting Medium (The Binding Site, Birmingham, U.K.). The slides were examined with a fluorescence microscope (Nikon) equipped with a B–2A filter (EX 450–490, DM 505, BA 520). Photographs were taken with a Nikon U-III, on Fujifilm Neopan Professional 1600 ASA film.

In control reactions, incubation with primary monoclonal antibody was omitted. The material was also checked for autofluorescence.

RESULTS

GALANTHUS NIVALIS

In tenuinucellar ovules of *G. nivalis* the embryo sac is extraordinarily large (200–500 μm), and the ovules containing it are wider than sterile ones. It was easy to distinguish the fertile and the sterile ovules by light microscopy. The micropylar parts of both types of ovules are anatomically similar, but after aniline blue staining

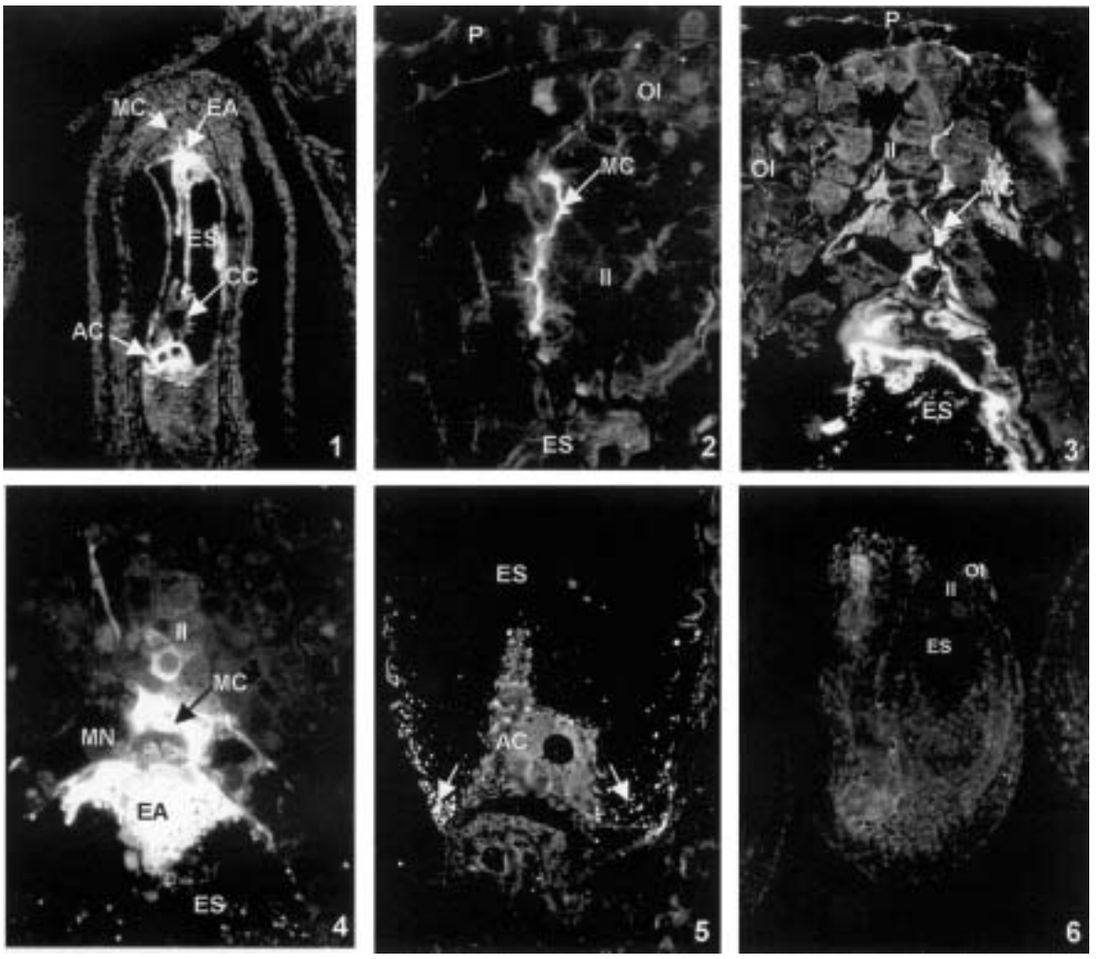

Fig. 1. *Galanthus nivalis* ovule 24 h after pollination, labelled with JIM 13 Mab. × 200. **Figs. 2, 3.** Micropylar part of *Galanthus nivalis* ovules 24 h after pollination, with strong fluorescence visible inside the micropylar canal. **Fig. 2.** Labelled with JIM 13 Mab. × 330. **Fig. 3**. Labelled with JIM 8 Mab. × 370. **Fig. 4.** Micropylar part of mature *Galtonia candicans* ovule before pollination, labelled with JIM 13 Mab, with strong fluorescence visible inside the egg apparatus and the micropylar canal. × 200. **Fig. 5.** Chalazal part of mature *Galtonia candicans* ovule before pollination, labelled with JIM 8 Mab; small points (arrows) visible at chalazal pole of embryo sac. × 370. **Fig. 6.** Control reaction, with monoclonal antibody omitted, in *Galtonia candicans* ovule. × 120. EA – egg apparatus; MC – micropylar canal; ES – embryo sac; CC – central cell; AC – antipodal cells; II – inner integument; OI – outer integument; P – placenta; MN – micropylar nucellus.

it was observed that the unfertile ovules were never penetrated by pollen tubes, while over 46% of the ovules containing embryo sac were fertilized 48 h after pollination.

AGP epitopes recognized by JIM 8 and JIM 13 Mab were not detectable by immunofluorescence in fertile ovules of *G. nivalis* collected in September at the stage of the fully formed embryo sac, nor in those taken from open but unpollinated (isolated) flowers.

Strong fluorescence after JIM 8 and JIM 13 Mab labelling appeared in the embryo sac of fertile ovules 24 h after cross-pollination. The patterns of fluorescence after labelling with both types of antibodies were similar. The most intense labelling was seen inside the cells of the egg apparatus, but fine strands of fluorescence were seen around the whole embryo sac, in the central cell and antipodal cells as well (Fig. 1). In somatic tissues, a positive reaction was detected in the intercellular spaces of the micropylar nucellus and along the micropylar canal formed by the inner integument, but was not seen in the cells surrounding this canal (Figs. 2, 3).

In sterile ovules of *G. nivalis*, the presence of AGP epitopes recognized by JIM 8 and JIM 13 Mab was not

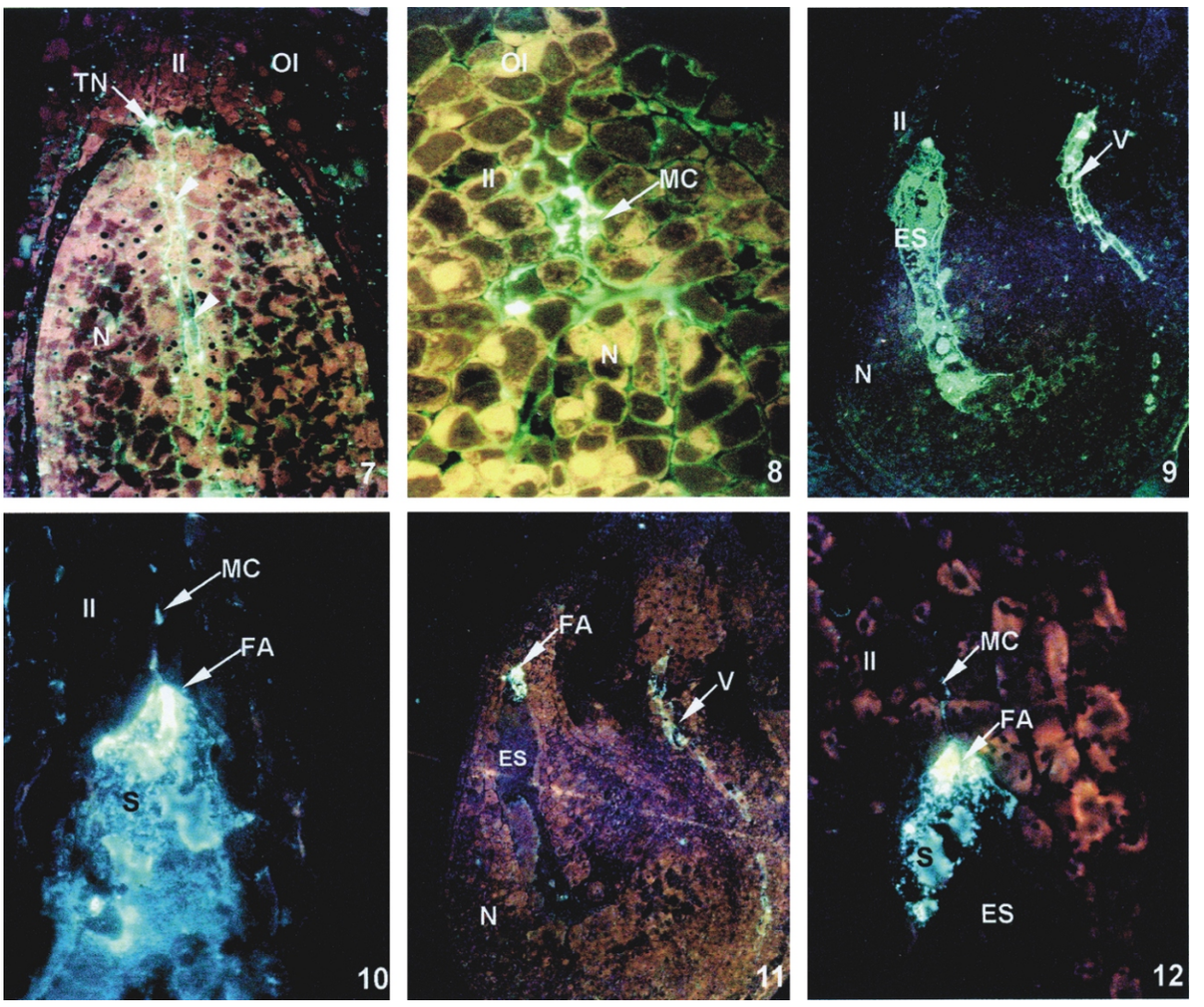

Fig. 7. Mature *Oenothera hookeri* ovule labelled with JIM 13 Mab, with fluorescence visible in the pathway leading from the top of the nucellus to the embryo sac (arrowheads). × 200. **Fig. 8.** Micropylar part of mature *Oenothera hookeri* ovule labelled with JIM 8 Mab, with fluorescence visible inside the micropylar canal formed by the inner integument. × 360. **Figs. 9, 10.** Mature *Sinapis alba* ovules labelled with JIM 13 Mab. **Fig. 9.** Fluorescence visible inside the whole embryo sac. × 200. **Fig. 10.** Intense fluorescence visible in the filiform apparatus. × 360. **Figs. 11, 12**. Mature ovules of *Sinapis alba* labelled with JIM 8 Mab, with fluorescence visible only on the micropylar pole of the embryo sac; Fig. 11. × 200, Fig. 12. × 360. MC – micropylar canal; ES – embryo sac; II - inner integument; OI – outer integument; N – nucellus; FA – filiform apparatus; V – vessels.

detected at any developmental stage. Sterile ovules from unpollinated or cross-pollinated flowers were checked several times during the flowering period to detect the presence of AGPs, but the results were always negative.

GALTONIA CANDICANS

Immunolabelling of young *G. candicans* ovules taken from flower buds at the stage of the 4-nucleate embryo sac revealed no AGP epitopes recognized by JIM 13 or JIM 8 in the developing embryo sac or in the somatic tissues of the ovule.

A clear positive reaction for AGP epitopes recognized by JIM 13 Mab appeared in ovules taken from freshly opened, unpollinated flowers. Similar results were obtained in ovules taken 24 h after cross-pollination. Strong fluorescence was visible inside the cells of the egg apparatus (Fig. 4). In other regions of the embryo sac, weak fluorescence was seen only in the central cell as small points. In somatic tissues, clear fluorescence after JIM 13 Mab labelling was detected

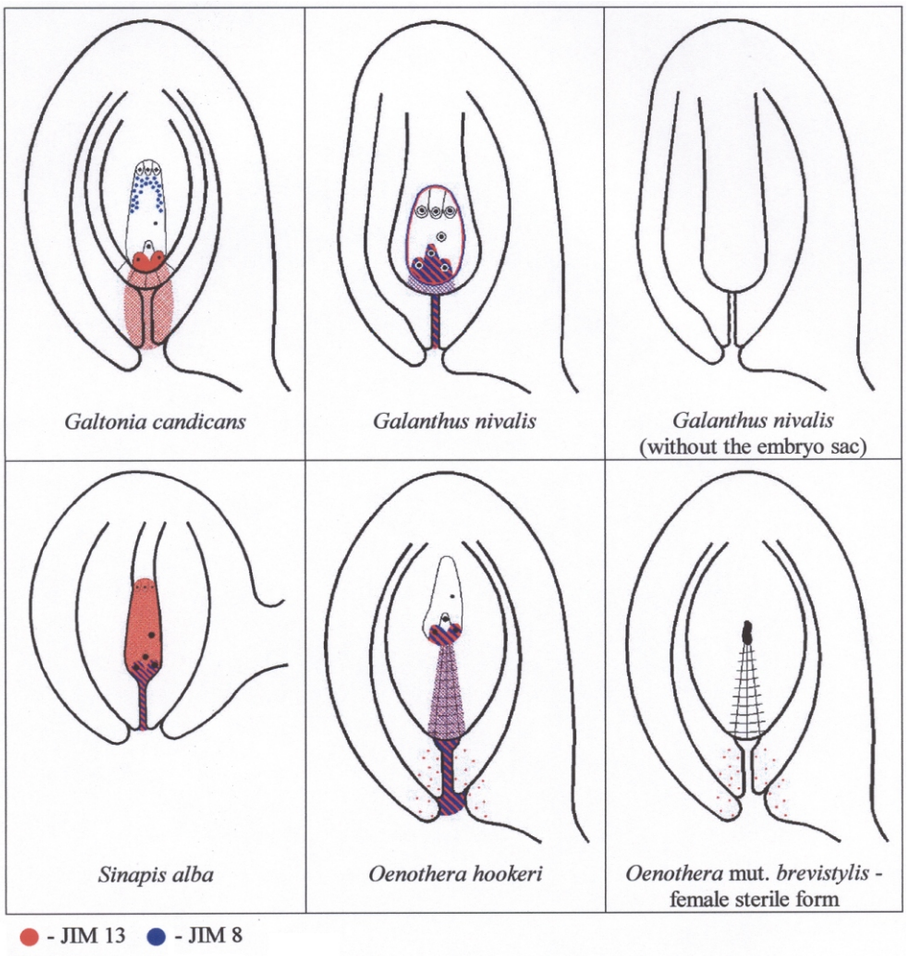

Fig. 13. Schematic drawing of localization of AGP antigenic determinants recognized by JIM 8 and JIM 13 Mab in mature ovules of *G. candicans*, *G. nivalis*, *S. alba*, *Oe. Hookeri* and *Oe.* mut. *brevistylis*.

in the cell cytoplasm and intercellular spaces of the micropylar nucellus and micropylar part of the inner integument, surrounding the micropylar canal (Fig. 4). In other parts of the somatic tissues of the ovule, epitopes recognized by JIM 13 Mab were not detected.

In contrast, immunolabelling of *G. candicans* ovules with JIM 8 Mab was not so clearly detectable as the epitope recognized by JIM 13 Mab. In mature ovules taken from open flowers, unpollinated or 24 h after pollination, JIM 8 Mab labelling produced only small points visible near the antipodal cells of the embryo sac (Fig. 5). In other parts of the ovule, fluorescence after JIM 8 Mab labelling was not visible at any developmental stage. In the control reactions, where the monoclonal antibody was omitted, no fluorescence was visible. An example of the control reaction in *G. candicans* is shown in Figure 6.

OENOTHERA HOOKERI

Young ovules of *Oe. hookeri* taken from buds at the stage of the developing embryo sac did not contain epitopes recognized by JIM 8 nor JIM 13 Mab.

The studied AGP epitopes appeared in mature *Oe. hookeri* ovules taken from freshly opened flowers, and were present in ovules taken 14 h after pollination as well. The results obtained after JIM 8 and JIM 13 Mab labelling were comparable. Strong fluorescence was visible at the micropylar pole of the embryo sac. In somatic tissues, AGPs were localized in intercellular spaces along the tract built of several cells lying between the micropylar pole of the embryo sac and the top of the nucellus. Fluorescence was especially distinct in the extracellular matrix filling the space between the micropylar top of the nucellus and the inner inte-

gument (Fig. 7). Fluorescence also occurred on the cell surface of the inner and outer integuments forming the micropylar canal (Fig. 8). After JIM 13 Mab labelling, small points inside the cytoplasm of the micropylar parts of the integuments also appeared (Fig. 7).

OENOTHERA mut. *BREVISTYLIS* – FEMALE STERILE FORM

In most of the *Oe.* mut. *brevistylis* ovules, the meiotic cells and the embryo sac did not develop, but in several ovules the generative cells started to develop but did not form tetrads or embryo sacs.

In young ovules of *Oe.* mut. *brevistylis*, the epitopes recognized by JIM 8 and JIM 13 Mab were not detected. Older ovules of the female sterile form *Oe.* mut. *brevistylis* were fixed and studied several times from anthesis to senescence, before and after pollination. At no developmental stage did JIM 8 and JIM 13 Mab labelling give positive results in the tract of cells leading from the micropylar canal through the nucellus to the degenerated embryo sac. Weak fluorescence was visible only in the micropylar part of integuments inside the cell cytoplasm and in cell walls after the reaction with JIM 13 Mab (not shown).

SINAPIS ALBA

In young ovules of *S. alba* taken from buds at the stage of the 4-nucleate embryo sac, the studied AGP epitopes were not detected in the embryo sac, nor in the integuments and degenerating micropylar nucellus. They were located only inside the vessels of the funiculus.

Strong fluorescence evidencing the presence of the epitope recognized by JIM 13 Mab appeared in mature embryo sacs of *S. alba* ovules taken from freshly opened flowers, and in ovules taken 6 h after pollination as well. A clear positive reaction was seen inside the whole embryo sac (Fig. 9), but especially intense fluorescence was seen in the filiform apparatus of the synergids (Fig. 10). Only the nuclei of the embryo sac showed no fluorescence.

The epitope recognized by JIM 8 Mab did not appear in the whole embryo sac; it was limited strictly to the cytoplasm of the synergids and the filiform apparatus (Fig. 11, 12). Both studied AGP epitopes were detected inside the micropylar canal as well, but were not present in the somatic cells surrounding the canal. Other parts of the mature ovules were devoid of AGP epitopes, except for the funiculus vessels.

DISCUSSION

Arabinogalactan proteins are considered to play an important role in interactions with other cell surface molecules and in cellular signaling. Negatively charged carbohydrate chains of AGPs can interact with other cell surface molecules, for example with pectins through Ca^{++} bridges. It is also possible that AGPs can associate with one another through oxidative cross-linking or ionic interactions (Showalter, 2001). The carbohydrate moieties of AGPs contain a rich array of biochemical information that could serve as signals consistent with oligosaccharide signaling in plants (Etzler, 1998; Showalter, 2001). AGPs possibly can act as signal molecules in sexual reproduction processes, especially at the final stage of the progamic phase when the pollen tube finds the micropyle of the ovule and when successful fertilization depends on recognition between the pollen tube and the mature embryo sac.

Several currently available monoclonal antibodies recognize different epitopes of AGPs which can be connected with the plasma membrane, bound to the cell wall, or secreted to the medium of suspension-cultured cells (Knox et al., 1991; Knox, 1997). Some of these epitopes, for example the epitope recognized by MAC 207 Mab, are abundant in most plant tissues. Pennell and Roberts (1990) obtained interesting results in *Pisum sativum* using MAC 207 antibody. This epitope is present in almost all somatic tissues of *Pisum sativum*, but disappears in cells connected with sexual reproduction: the embryo sac and the nucellus of the mature ovule, and the tapetum and sporogenic tissue in the anther. Those authors suggested that the molecules recognized by MAC 207, which at the beginning are especially abundant on the cell surface, disappear to make room for other substances important for the sexual reproduction process.

In *Arabidopsis thaliana*, Acosta-Garcia and Vielle-Calzada (2004) showed that *AGP18*, a gene encoding a classic arabinogalactan protein, is expressed in cells that spatially and temporally define the sporophyte-to-gametophyte transition, and during early stages of seed development. In *AGP18*-silenced plants, the functional megaspore fails to enlarge and mitotically divide, indicating that this gene is essential to the initiation of female gametogenesis in *Arabidopsis*.

Several studies have documented the presence of epitopes recognized by JIM 8 and JIM 13 in cells connected with sexual reproduction. The epitope recognized by JIM 13 was found in exudates from epidermal cells surrounding the canal in the open style of *Lilium*, but was not present in other cells of the style (Jauh and Lord, 1996). Epitopes recognized by JIM 8 Mab were found on the sperm cell surface in *Brassica napus*, *Brassica campestris* and *Arabidopsis thaliana* (Pennell et al., 1991; van Aelst and van Went, 1992; Southworth and Kwiatkowski, 1996). In mature crassinucellar ovules of *Amaranthus hypochondriacus*, the presence of JIM 8 and JIM 13 epitopes was shown in the egg apparatus and in the cells of the nucellus and integuments lining the pollen tube growth pathway (Coimbra and Salema, 1997). The presence of JIM 8 epitope was also shown in the egg cell, synergids and inner integument of *Brassica napus* ovules (Pennell et al., 1991).

In all the types of fertile and mature ovules studied in the present work, the epitopes recognized by JIM 8 or JIM 13 Mab were marked especially distinctly in the egg apparatus and the pathway of pollen tube growth, although some differences in their distribution were observed (Fig. 13). In *G. nivalis*, *Oe. hookeri* and *S. alba*, both studied epitopes of AGPs were present in the tissues lying on the pathway of pollen tube growth, while in *G. candicans* only the epitope recognized by JIM 13 Mab was found on that pathway. The localization of the studied AGP epitopes was not connected with a specific tissue or type of cell but with the position of the cells. This phenomenon is well known from studies on the localization of different AGP epitopes during root tissue formation (Knox et al., 1989, 1991) and in somatic embryogenesis (Serpe and Nothnagel, 1996, 1999).

The timing of the appearance of JIM 8 and JIM 13 epitopes in the studied ovules suggests that they indicate the ovule's receptivity. Depending on the plant species, the studied epitopes appeared in ovules from opening flowers regardless of whether they were pollinated (in *G. candicans*, *Oe. hookeri* and *S. alba*), or only in ovules from pollinated flowers (*G. nivalis*). This phenomenon may be connected with the biology of flowering of *G. nivalis*, in which the unpollinated flowers can stay open for over three weeks, depending on the weather, while after pollination the flowers whither in three days. In this species it has also been shown that pollination stimulates embryo sac enlargement, and in some ovules even their further development (Chudzik et al., 2002).

In all the studied receptive ovules, the AGP epitopes were present inside the embryo sac, mainly in the cells of the egg apparatus. Presumably they are synthesized inside gametophyte cells and can be secreted to the extracellular matrix filling the micropylar canal. In *G. candicans* and *Oe. hookeri*, fluorescence was detected inside the somatic cells of the micropylar tissues, but observed only in ovules containing mature embryo sacs. No AGP epitopes recognized by JIM 8 and JIM 13 were detected in somatic micropylar tissues in ovules of *G. nivalis* and *Oe.* mut. *brevistylis*, both without embryo sacs. The somatic tissues of the micropylar part of integuments and nucelli actively participate in preparing suitable conditions for pollen tube growth, but their activity evidently depends on stimulation of the mature embryo sac (Chudzik, 2003). Several specific histochemical and ultrastructural changes have been described in somatic micropylar tissues of the maturing ovule, preparing a suitable environment for pollen tube growth (Chudzik and Śnieżko, 1999a,b, 2003; Chudzik, 2002, 2003). The appearance of specific AGP epitopes is one of these phenomena, but the timing and localization of AGP expression is restricted mostly to the time of highest ovule receptivity and to the pathway of pollen tube growth.

ACKNOWLEDGEMENTS

The authors are grateful to Professor Keith Roberts of the Department of Cell Biology, John Innes Centre (U.K.), for kindly providing the monoclonal antibodies JIM 8 and JIM 13.

REFERENCES

ACOSTA-GARCIA G, and VIELLE-CALZADA JP. 2004. A classical arabinogalactan protein is essential for the initiation of female gametogenesis in *Arabidopsis*. *The Plant Cell* 16: 2614–2628.

CHEN C-G, MAU S-L, and CLARKE AE. 1993. Nucleotide sequence and style specific expression of a novel proline-rich protein gene from *Nicotiana alata*. *Plant Molecular Biology* 21: 391–395.

CHEUNG AY, MAY B, KAWATA EE, GU Q, and WU H-M 1993. Characterization of cDNAs for stylar transmitting tissue-specific proline-rich proteins in tobacco. *Plant Journal* 3: 151–160.

CHEUNG AY, WANG H, and WU H-M. 1995. A floral transmitting tissue-specific glycoprotein attracts pollen tubes and stimulates their growth. *Cell* 82: 383–393.

CHUDZIK B. 2002. Procesy determinujące receptywność zalążków o różnej budowie anatomicznej u roślin z rodzin: Amaryllidaceae, Brassicaceae, Liliaceae i Oenotheraceae. Ph.D. dissertation, Maria Curie-Skłodowska University, Lublin, Poland (in Polish)

CHUDZIK B. 2003. Rozwój i dojrzewanie żeńskich organów generatywnych u *Galtonia candicans* (Liliaceae). *Annales Universitatis Curie-Skłodowska, Sectio EEE Horticultura* 13: 1–8.

CHUDZIK B, and ŚNIEŻKO R. 1997. Testing the fertility in ovules of *Oenothera hookeri*, *Oe.* mut. *brevistylis* – female sterile form, *Capsella bursa-pastoris* and *Sisymbrium loeselii* by induction of callose fluorescence. *Bulletin of the Polish Academy of Sciences, Biological Sciences* 45: 283–288.

CHUDZIK B, and ŚNIEŻKO R. 1999a. Histochemical features signaling receptivity of ovules of *Oenothera hookeri* de Vries and *Oe.* mut. *brevistylis*. *Acta Biologica Cracoviensia Series Botanica* 41: 119–126.

CHUDZIK B, and ŚNIEŻKO R. 1999b. Przejawy receptywności zalążków *Brassica napus* L. i *Sinapis alba* L. *Bibliotheca Fragmenta Agronomica* 6: 57–66.

CHUDZIK B, and ŚNIEŻKO R. 2003. Calcium ions presence as a trait of receptivity in tenuinucellar ovules of *Galanthus nivalis* L. *Acta Biologica Cracoviensia Series Botanica* 45/1: 133–141.

CHUDZIK B, ŚNIEŻKO R, and SZAUB J. 2002. Biologia kwitnienia *Galanthus nivalis* L. (Amaryllidaceae). *Annales Universitatis Mariae Curie-Skłodowska, Lublin – Polonia, Sectio EEE Horticultura* 10: 1–10.

CLARKE AE, ANDERSON RL, and STONE BA. 1979. Form and function of arabinogalactans and arabinogalactan-proteins. *Phytochemistry* 18: 521–540.

COIMBRA S, and SALEMA R. 1997. Immunolocalization of arabinogalactan proteins in *Amaranthus hypochondriacus* L. ovules. *Protoplasma* 199: 75–82.

COIMBRA S, and DUARTE C. 2003. Arabinogalactan proteins may facilitate the movement of pollen tubes from the stigma to the ovules in *Actinidia deliciosa* and *Amaranthus hypochondriacus*. *Euphytica* 133: 171–178.

DU H, CLARKE AE, and BACIC A. 1996. Arabinogalactan-proteins: a class of extracellular matrix proteoglycans involved in plant growth and development. *Trends in Cell Biology* 6: 411–414.

ETZLER ME. 1998. Oligosaccharide signalling of plant cells. *Journal of Cell Biochemistry, Suppl*. 30–31: 123–128.

FINCHER GB, STONE BA, and CLARKE AE. 1983. Arabinogalactan-proteins: Structure, biosynthesis and function. *Annual Review of Plant Physiology* 34: 47–70.

GANE AM, CLARKE AE, and BACIC A. 1995a. Localisation and expression of arabinogalactan-proteins in the ovaries of *Nicotiana alata* Link and Otto. *Sexual Plant Reproduction* 8: 278–282.

GANE AM, CRAIK D, MUNRO SLA, HOWLETT GJ, CLARKE AE, and BACIC A. 1995b. Structural analysis of the carbohydrate moiety of arabinogalactan-proteins from stigmas and styles of *Nicotiana alata*. *Carbohydrate Research* 277: 67–85.

GLEESON PA, and CLARKE AE. 1979. Structural studies on the major component of *Gladiolus* style mucilage, an arabinogalactan-protein. *Biochemical Journal* 181: 607–621.

GLEESON PA, and CLARKE AE. 1980. Arabinogalactans of sexual and somatic tissues of *Gladiolus* and *Lillium*. *Phytochemistry* 19: 1777–1782.

GOLDMAN DE MHS, PEZZOTTI M, SEURINCK J, and MARIANI C. 1992. Developmental expression of tobacco pistil-specific genes encoding novel extensin-like proteins. *Plant Cell* 4: 1041–1051.

JAUH GY, and LORD EM. 1996. Localization of pectins and arabinogalactan-proteins in lily (*Lilium longiflorum* L.) pollen tube and style, and their possible roles in pollination. *Planta* 199: 251–261.

KNOX JP. 1995. Developmentally regulated proteoglycans and glycoproteins of the plant cell surface. *FASEB Journal* 9: 1004–1012.

KNOX JP. 1996. Arabinogalactan-proteins: Developmentally regulated proteoglycans of the plant cell surface. In: Smallwood M, Knox JP, Bowles DJ [eds.], *Membranes: specialized functions in plants*, 93–102. Bios Scientific, Oxford.

KNOX JP. 1997. The use of antibodies to study the architecture and developmental regulation of plant cell walls. *International Review of Cytology* 171: 79–120.

KNOX JP. 1999. Intriguing, complex and everywhere: getting to grips with arabinogalactan-proteins. *Trends in Plant Science* 4: 123–125.

KNOX JP, DAY S, ROBERTS K. 1989. A set of cell surface glycoproteins form an early marker of cell position, but not cell type, in the root apical meristem of *Daucus carota* L. *Development* 106: 47–56.

KNOX JP, LINSTEAD PJ, PEART J, COOPER C, and ROBERTS K. 1991. Developmentally regulated epitopes of cell surface arabinogalactan proteins and their relation to root tissue pattern formation. *Plant Journal* 1: 317–326.

LIND JL, BACIC A, CLARKE AE, and ANDERSON MA. 1994. A style-specific hydroxyproline-rich glycoprotein with properties of both extensis and arabinogalactan proteins. *Plant Journal* 6: 491–502.

PENNELL RI, and ROBERTS K. 1990. Sexual development in the pea is presaged by altered expression of arabinogalactan protein. *Nature* 344: 547–549.

PENNELL RI, JANNICHE L, KJELLBOM P, SCOFIELD GN, PEART JM, and ROBERTS K. 1991. Developmental regulation of a plasma membrane arabinogalactan protein epitope in oilseed rape flowers. *Plant Cell* 3: 1317–1326.

SEDGLEY M, and CLARKE AE. 1986. Immuno-gold localisation of arabinogalactan protein in the developing style of *Nicotiana alata*. *Nordic Journal of Botany* 6: 591–598.

SEDGLEY M, BLESING MA, BONIG I, ANDERSON MA, and CLARKE AE. 1985. Arabinogalactan proteins are localised extracellularly in the transmitting tissue of *Nicotiana alata* Link and Otto, an ornamental tobacco. *Micron Microscopica Acta* 16: 247–254.

SERPE MD, and NOTHNAGEL EA. 1996. Heterogeneity of arabinogalactan-proteins on the plasma membrane of *Rosa* cells. *Plant Physiology* 112: 1261–1271.

SERPE MD, and NOTHNAGEL EA. 1999. Arabinogalactan-proteins in the multiple domains of the plant cell surface. *Advances in Botanical Research* 30: 207–289.

SHOWALTER AM. 2001. Arabinogalactan-proteins: structure, expression and function. *Cellular and Molecular Life Sciences* 58: 1399–1417.

SOMMER-KNUDSEN J, CLARKE AE, and BACIC A. 1996. A galactose-rich, cell-wall glycoprotein from styles of *Nicotiana alata*. *Plant Journal* 9: 71–83.

SOUTHWORTH D, and KWIATKOWSKI S. 1996. Arabinogalactan proteins at the cell surface of *Brassica* sperm and *Lilium* sperm and generative cells. *Sexual Plant Reproduction* 9: 269–272.

VAN AELST AC, and VAN WENT JL. 1992. Ultrastructural immuno-localization of pectins and glycoproteins in *Arabidopsis thaliana* pollen grains. *Protoplasma* 168: 14–19.

WEBB MC, and WILLIAMS EG. 1988. The pollen tube pathway in the pistil of *Lycopersicon peruvianum*. *Annals of Botany* 61: 415–423.

WU H-M, WANG H, and CHEUNG AY. 1995. A pollen tube growth stimulatory glycoprotein is deglycosylated by pollen tubes and displays a glycosylation gradient in the flower. *Cell* 82: 393–403.

YARIV J, LIS H, and KATCHALSKI E. 1967. Precipitation of arabic acid and some seed polysaccharides by glucosylphenylazo dyes. *Biochemical Journal* 105: 1c–2c.

YATES EA, VALDOR J-F, HASLAM SM, MORRIS HR, DELL A, MACKIE W, and KNOX JP. 1996. Characterization of carbohydrate structural features recognized by anti-arabinogalactan-protein monoclonal antibodies. *Glycobiology* 6: 131–139.