Isolation of de-exined pollen and cytological studies of the pollen intines of *Pinus bungeana* Zucc. Ex Endl. and *Picea wilsonii* Mast

Kefeng Fang\(^a,b\), Younian Wang\(^a\), Tongquan Yu\(^a\), Zhang Lingyun\(^b,c\), František Baluška\(^d\), Jozef Šamaj\(^d\), Lin Jinxing\(^b,*\)

\(^a\)Key Laboratory of New Agricultural Technology and Application in Beijing, Beijing University of Agriculture, Beijing 102206, China
\(^b\)Key Laboratory of Photosynthesis and Molecular Environmental Physiology, Institute of Botany, Chinese Academy of Sciences, Xiangshan, Beijing 100093, China
\(^c\)College of Natural Resource and Environment, Beijing Forestry University, Beijing 100083, China
\(^d\)Institute of Cellular and Molecular Botany, University of Bonn, Kirschallee 1, D-53115 Bonn, Germany

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**Abstract**

To study the cytological and biochemical characteristics of intine, pollen deprived of exine, or de-exined pollen, was isolated from the gymnosperms *Pinus bungeana* and *Picea wilsonii*. The factors influencing the isolation rate were examined. Cellulose, callose, pectin, and arabinogalactan proteins (AGPs) were localized in this material using fluorescent probes, and components of the isolated intine were further analyzed by Fourier transform infrared (FTIR) microspectroscopy. The isolation protocol was repeatable and reliable. Cellulose was found to be evenly distributed on the surface of the intine, as indicated by strong calcofluor White ST (CW) fluorescence, and aniline blue staining revealed that callose was present on the intine of *P. bungeana* but not on that of *P. wilsonii*. Immunolabeling revealed that acidic pectin epitopes recognized by the monoclonal antibody JIM5 were present on the pollen intine, as well as esterified pectin recognized by the monoclonal antibody JIM7, and AGPs recognized by the LM2 antibody. Two lectin binding sites, the concanavalin agglutinin (Con A) and soybean agglutinin (SBA) binding sites, were present on the intine surface, but no wheat germ agglutinin (WGA) binding sites were detectable. These results were confirmed by FTIR analysis.

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**Introduction**

Pollen grain of flowering plants is encased by a tough, rigid outer coat, the exine. Underlying the exine is a layer of polysaccharide-rich material, the intine, which envelopes the membrane-enclosed male gametophyte (Loewus et al., 1985; Singh, 1978). In general, the pollen wall of gymnosperms is formed according to the following process: in the beginning, exine material – sporopollenin – is deposited on the surface of the pollen, the callosic outer intine is subsequently formed under the exine, and the inner intine composed by polysaccharides that successively appear (Rowley et al., 2000).
“De-exined pollen” is the term used to describe pollen deprived of the exine, a state between an entire pollen grain and a pollen protoplast. Pollen is an ideal system for certain biological studies due to its haploid state, the large scales that are possible, and its ease of manipulation. De-exined pollen is covered only by the intine, providing an ideal material for studying the structure of the germ aperture and the roles of the exine and intine during pollen germination and tube growth. The unique characteristics of de-exined pollen make it a desirable system for pollen biological studies. Isolation and cytological studies of viable de-exined pollen have been performed in angiosperms, including Nicotiana tabacum (Xia et al., 1996), Brassica (Xu et al., 1996, 1997), and Lilium longiflorum (Loewus et al., 1985). In gymnosperms, exine detaches from the intine of some genera including Taxus (Duhoux, 1982) and Ephedra (El-Ghazaly et al., 1998) when pollen is hydrated and the protoplasts and intine swell, as it occurs in the Picea wilsonii and P. bungeana. So far, no method has been available for the isolation of viable de-exined pollen of P. bungeana and P. wilsonii, much less for cytological studies or gene transformation. It is crucial to establish an efficient protocol for the isolation of de-exined pollen of these conifers in order to provide materials for cytological studies.

Arabinogalactan proteins (AGPs), which can be detected in a number of different plant organs, are involved in such processes as programmed cell death and vegetative, reproductive, and cellular growth and development (Showalter, 2001). And in general, lectin-mediated reactions and processes depend on the specific binding of the lectin to glycoconjugate receptors, referred to as glycoconjugates, which possess a carbohydrate moiety with a structure complementary to that of the lectin receptors, namely glycoproteins, glycolipids, and oligosaccharides (Peumans and Van Damme, 1995).

Because a pure intine is difficult to isolate, because it is covered by the enzyme-resistant exine, which is mainly composed by sporopollenin, its isolation has been successful in only a few angiosperms, including tobacco and Brassica (Xia et al., 1996; Xu et al., 1996). Heslop-Harrison and Heslop-Harrison (1982) introduced an intine-ghost method to remove exine and protoplast by chemicals. However, under this condition the remaining intine was mainly composed by cellulose with other components destroyed and therefore it could not represent the in vivo nature.

The objective of the present study was to establish a simple and efficient protocol for the preparation of large numbers of living de-exined P. bungeana and P. wilsonii pollen and to address the main factors that affect its preparation. Based on these data, a series of cytological studies was conducted in an attempt to verify the intactness of the intine and to gain informations as to its role. We present a detailed study on the isolation of de-exined pollen and cytological studies of the pollen intine in gymnosperms with the intention of comparing these results to those of angiosperms. The cytological studies focused on the distribution of cellulose, pectins, callose, lectin binding sites, and AGPs at the intine surface. In addition, Fourier transform infrared (FTIR) microspectroscopy was used to analyze the components of the P. bungeana and P. wilsonii pollen intines.

Materials and methods

Plant materials

Pollen was collected from trees of P. wilsonii Mast. and P. bungeana Zucc. ex Endl. growing in the Botanical Garden of the Institute of Botany, Chinese Academy Sciences on 5 May and 18 April, 2005, respectively, when the majority of the cones were ripe and pollinating. The collected pollen was left on Petri dishes for several days at room temperature to dehydrate. Dried pollen grains were stored at −20°C for preparation.

Isolation protocol for de-exined pollen

Before treatment, the pollen was allowed to adjust to room temperature for 30 min, and was put into 12–15% sucrose solution supplemented with 0.01% H$_3$BO$_3$, 0.01% CaCl$_2$ (base medium), 1–2% cellulase R-10, 0.5–1.5% macerozyme R-10 and incubated for 0.5 h to be rehydrated. Then the material was washed with the base medium for at least three times to remove the enzymes. Then droplets containing pollen grains were grinded gently with a coverslip by suitable pressure so that de-exined pollen were released. Some living isolated de-exined pollen were used freshly, whereas the rest was fixed in 3% freshly prepared paraformaldehyde in 0.1 M PBS buffer (pH 7.2) for 30 min, followed by three washes in pure PBS buffer.

Fluorescein diacetate (FDA, Sigma) was dissolved in acetone to produce a 1mg/ml stock solution. For viability tests, the FDA stock was added to the medium to a final concentration of 1 mg/ml.

Localization of cellulose

Calcofluor White ST (CW, Sigma) was applied to determine the presence of cellulose on the surface of the isolated de-exined pollen. After 15 min of staining, the de-exined pollen was collected and transferred into dye-free medium.
Immunolabeling of pectins

The freshly or fixed isolated de-exined pollen was incubated at room temperature for 1 h with the primary antibodies JIM5 or JIM7 (1:5 concentration), specific against acidic pectin and esterified pectin, respectively. Thereafter it was rinsed three times (5 min each) in sucrose solution or PBS. Samples were incubated then with a second antibody, fluorescein isothiocyanate (FITC), labeled with goat anti-rat IgG antiserum (Sigma), and diluted 1:100 with PBS (pH 7.2) at room temperature for 1 h. After a final rinse series in PBS, the samples were mounted on slides and observed and photographed with LSCM (Zeiss, LSM 510 META, Germany) with excitation at 488 nm and emission at 522 nm.

Aniline blue staining of callose

Freshly isolated de-exined pollen was incubated with 0.1% decolorized aniline blue in 0.1 M PBS buffer (pH 8.2) for approximately 5 min, immediately mounted and photographed with a laser scanning confocal microscope (LSCM) (Zeiss, LSM 510 META, Germany). The samples were excited at 488 nm with a 25 mW argon ion laser and emitted radiation at 515 nm. To decrease unspecific staining by aniline blue, toluidine blue O (0.5% in 0.1 M PBS buffer, pH 7.0) was used after aniline blue staining.

Immunolabeling of arabinogalactan proteins (AGPs)

The immunolabeling protocol followed the method described by Pennell et al. (1991). Living or fixed samples were incubated with the monoclonal antibodies (diluted 1:5 in BSA) overnight at 4 °C. After washing, the materials were incubated with an anti-rat immunoglobulin antibody conjugated with FITC (diluted 1:100 in BSA), and observed with a Zeiss fluorescence microscope. The presence of a positive reaction between the antibody and its epitope was indicated by the presence of a yellow-green stain due to the polyclonal antibody. Controls were prepared by excluding the incubation with monoclonal antibodies.

FTIR microspectroscopy

Isolated pure pollen intine was fully washed with deionized water for five times and then dried in a layer on a barium fluoride window (13 mm diameter × 2 mm). FTIR spectra were recorded on a Perkin-Elmer Cetus MAGNA 750 FTIR spectrometer (Nicolet Corporation, Tokyo, Japan) equipped with a mercury–cadmium–telluride (MCT) detector, and the Perkin-Elmer Cetus microscope interfaced to a personal computer. An area of approximately 500 × 500 μm was selected for FTIR analysis. The acquisition parameters were 8 cm⁻¹ resolution, 128 co-added interferograms, and normalized to obtain relative absorbance.

Localization of lectin binding sites

The labeling protocol was according to the method described by Fang et al. (2005) with some modifications. Three selected lectins, namely concanavalin agglutinin (Con A), wheat germ agglutinin (WGA) and soybean agglutinin (SBA), conjugated with FITC functioned as probes to localize the distribution of their binding sites. Around 0.01% calcium solution was added to improve the labeling (Fang et al., 2005).

Results

Establishment of a protocol to isolate de-exined gymnosperm pollen

A rapid, efficient, and repeatable protocol was established to obtain a large number of living de-exined P. bungeana and P. wilsonii pollen. Multiple factors were essential for the isolation of de-exined pollen. The most suitable conditions to achieve the isolation in both species were by the application of an enzyme mixture consisting of 1% cellulase R-10, 0.8% macerozyme R-10, and 15% or 12% sucrose to keep osmotic stress, at pH 5.8, with gentle agitation for 0.5 h at 24 °C. The freshly isolated de-exined pollen was oval-shaped, as are pollen grains in vivo, no exine was present on the surface, and P. bungeana de-exined pollen exhibited the obvious callosic components (Fig. 1a). The tube cell was near the distal pole and the body cell and stalk cell were located at the proximal pole of the pollen (cf. Runions et al., 1999; Singh, 1978). Strong FDA fluorescence demonstrated the viability of the isolated de-exined pollen, while the entire pollen grain could not emit FDA fluorescence owing to the existence of the exine.

The isolation yield of de-exined pollen depended on the enzymatic treatment and the pressure used during grinding. The examined enzymatic treatment variables were the enzyme concentration, the ratio of the different enzymes, and the incubation time. Isolation of de-exined...
pollen was most successful, when the enzyme concentration was low (1%) and the enzymatic treatment incubation time was short (up to 0.5 h). Varying the concentrations of cellulase and macerozyme while holding other parameters constant revealed that 1% cellulase and 0.8% macerozyme were the most suitable concentrations for isolating de-exined pollen, with the highest isolation rate and viability, resulting in isolation rates of up to 70%. Varying the incubation time while holding the other parameters constant showed that 0.5 h was the most suitable incubation time for isolation of de-exined pollen. Furthermore, the pressure used in grinding was critical for the successful isolation of de-exined pollen. Higher pressures resulted in the de-exined pollen being broken or not viable, while with too low level of grinding pressure, de-exined pollen could not be detached from the exine.

The de-exined pollen of the two species differed in their morphological characteristics, including shape, volume, and components. De-exined *P. bungeana* pollen was triangle shaped or ellipse shaped with the intine (callose) in a form corresponding to the two sacci. In contrast, de-exined *P. wilsonii* pollen was ellipse shaped and largely without the callose wings (Fig. 1b).

**Cytological studies**

Cytological studies were conducted on the intine surface of the isolated de-exined pollen of both gymnosperm species to localize cellulose, callose, pectin, AGP, and lectin binding sites. In addition, the components of the complete intine were analyzed by FTIR spectrometry.

**Localization of cellulose**

Strong fluorescence from CW indicated the presence of cellulose on the intine surface of isolated *P. bungeana* de-exined pollen. Concentrated (dense) fluorescence appeared at the sites where stalk cells were located, and obvious CW fluorescence was also present on the surface of *P. bungeana* generative cells (Fig. 2a).

In contrast, CW fluorescence on the *P. wilsonii* intine surface was not stronger than on other parts (Fig. 2b). Fluorescence was also detected on the body cell surface. The presence of cellulose confirmed that the isolated de-exined pollen was not dramatically affected by the enzymatic treatment.

**Localization of callose**

Fluorescence labeling with decolorized aniline blue clearly showed that callose was present on the *P. bungeana* pollen intine in the shape of two wings (Fig. 2c), but was not labeled on the *P. wilsonii* intine. On freshly isolated pollen intine, strong fluorescence intensity was predominantly present at the site underneath the sacci, with no or little fluorescence detected on other regions of the intine surface. The fluorescence intensity declined gradually from the sacci toward the distal polar end and the proximal end. On the *P. wilsonii* intine surface, decolorized aniline did not stain, indicating the absence of callose.

**Immunocytochemical localization of pectins**

Fluorescence due to JIM5 labeling revealed a relatively homogeneous distribution of acidic pectin all over the intine surface of living de-exined *P. bungeana* pollen (Fig. 2d). In contrast, in living de-exined pollen of *P. wilsonii* stronger JIM5 fluorescence was present on the surface of the proximal pole (Fig. 2e).

The JIM7 fluorescence in *P. bungeana* de-exined pollen was limited to the region around the germ furrow, with the rest of the intine surface emitting fluorescence that was either faint or too low to be detected (Fig. 2f). However, in *P. wilsonii* de-exined pollen, JIM7 fluorescence was detected on the entire surface of the pollen intine (Fig. 2g).

**Localization of arabinogalactan protein**

The LM2 antibody was used to localize AGPs in these samples. A strong, smooth fluorescent signal was detected on the entire surface of the *P. bungeana* pollen intine (Fig. 2h). The intine surface of de-exined *P. wilsonii* pollen also emitted fluorescence, with the strongest signal at the site where the stalk cell is located and weaker signals in other regions (Fig. 2i).

**Localization of lectin binding sites**

Three lectins, Con A, WGA and SBA, each conjugated with FITC were used as fluorescent probes to localize their binding sites in de-exined pollen. Con A
and SBA binding sites were labeled on the intines of pollen of the two species. In contrast, no fluorescence was emitted by intines of either species labeled with FITC-WGA, indicating that no WGA binding sites were present or that their levels were too low to be detected on the pollen intine surfaces.

In *P. bungeana* pollen, Con A binding sites were present on the intine surfaces, but no fluorescence was observed on the callose portions (Fig. 3a). In contrast, Con A binding sites were detected over the entire intine of *P. wilsonii* pollen (Fig. 3b).

SBA binding sites were localized evenly on the *P. bungeana* pollen intine (Fig. 3c), whereas in *P. wilsonii*, SBA fluorescence was distributed non-uniformly, with stronger fluorescence on the surface of the proximal pole and weaker fluorescence on the surface of the distal pole (Fig. 3d).

Fixation of the isolated de-exined *P. bungeana* pollen in freshly prepared 3% paraformaldehyde resulted in much stronger JIM5 fluorescence on the intine surface, with the fluorescence concentrated on the sides where the two sacci are located (Fig. 3e), although more JIM7 fluorescence was localized at the site corresponding to the prothallial cell (Fig. 3f). Compared to living tissues, fixed materials emitted stronger fluorescence when the parameters of the labeling protocol were held constant.

**FTIR analysis**

Entire intines were successfully isolated from *P. bungeana* (Fig. 4a) and *P. wilsonii* (Fig. 4b) pollen, and the intines were analyzed by FTIR microspectroscopy. A typical FTIR spectrum obtained from *P. bungeana* pollen intine is shown in Fig. 5. Amide-stretching protein bands occurred at 1551 cm\(^{-1}\) (McCann et al., 1994), phenolics appeared at 1615 cm\(^{-1}\) (Williams and Fleming, 1980), and pectin peaks appeared at around 1733 and 1105 cm\(^{-1}\), with esterified pectin absorbing at 1733 cm\(^{-1}\) (McCann et al., 1994; Morikawa et al., 1978), and polysaccharides absorbing at 1200–900 cm\(^{-1}\) (Hori
The FTIR spectra suggested a higher content of arabinogalactan because of the higher intensity of the peak at \(1080\, \text{cm}^{-1}\). Lignin and carboxylic acid peaks at around \(1424\) and \(1243\, \text{cm}^{-1}\) (Pandey and Pitman, 2003), respectively, and cellulose and hemicellulose peaks at around \(1370, 1316, 1157,\) and \(1059\, \text{cm}^{-1}\) (Pandey and Pitman, 2003; Zeier and Schreiber, 1999) were clearly present in the spectrum of the \(P.\) bungeana pollen intine. The highest peak at \(1058\, \text{cm}^{-1}\) indicated the presence of a high content of cellulose.

The FTIR spectrum derived from the \(P.\) wilsonii pollen intine was similar to that of \(P.\) bungeana, except that the absorbance was greater than that of the latter. In the \(P.\) wilsonii FTIR spectrum, phenolics appeared at \(1620\) and \(1515\, \text{cm}^{-1}\) (McCann et al., 1994; Williams and Fleming, 1980), saturated esters absorbed at \(1735\, \text{cm}^{-1}\) (McCann et al., 1994; Morikawa et al., 1978), and polysaccharide absorbed at \(1200–900\, \text{cm}^{-1}\) (Hori and Sugiyama, 2003). This spectrum suggested a high content of arabinogalactan.
content of arabinogalactan because of the high intensity of the peak at 1080 cm\(^{-1}\). Lignin and carboxylic acid peaks also appeared at around 1418 and 1244 cm\(^{-1}\) (Pandey and Pitman, 2003), and cellulose and hemicellulose peaks appeared at around 1371, 1155, and 1058 cm\(^{-1}\) (Pandey and Pitman, 2003; Zeier and Schreiber, 1999). These results support the results of the above cytological studies (Fig. 6).

Comparison of optimal isolation protocols and results of the cytological studies of the two species

Similarities
There were some similarities in the results of the cytological studies for the de-exined pollen of the two species, including the localization of binding sites for Con A, SBA, and unesterified pectin. CW fluorescence was also detected on generative cell walls of both species. In addition, Con A, SBA, JIM5, and JIM7 labeled the pollen intines of the two species. Finally, the optimal protocols for the isolation of de-exined pollen from the two species were similar.

Differences
De-exined \(P. \) bungeana pollen was smaller than that of \(P. \) wilsonii, with the diameter of the former half that of the latter. In addition, callose was present on the intine of de-exined \(P. \) bungeana pollen, but absent from that of \(P. \) wilsonii.

Discussion

Establishment of isolation protocol
In an effort to bridge the gap between gymnosperm and angiosperm pollen tube manipulation, we established a protocol for the isolation of de-exined gymnosperm pollen on the basis of well-known methods used for angiosperms. The isolation protocol established in this investigation is simple, time saving, and efficient, with a greater yield obtained than that reported for angiosperms (Xia et al., 1996). The protocol is simple, not requiring heat, cold, or osmotic shock treatments, unlike the protocol used for angiosperms. Loewus et al. (1985) reported that pollen sporoplasts could be released from Lilium longiflorum pollen by 4-Methylmorpholine \(N\)-oxide monohydrate (MMNO \(\cdot\) \(H_2\)\(O\)) treatment at high temperature, which might have no viability. Southworth (1988) described the isolation and purification of exines from several hydrated and autoclaved angiosperm pollen, which resulted in dead products. In addition, the method described in the present research is time saving, with an enzymatic treatment of 0.5 h followed by grinding, in contrast to an enzymatic treatment lasting from 0.5 h to several days before release of de-exined angiosperm pollen. The protocol is efficient, with isolation rates up to 80%. Finally, the isolation method is more convenient than the complex one of angiosperms. FDA fluorescence information supported the results of Nepi et al. (2005), which demonstrated fluorescence only with de-exined pollen but not with entire ones. The isolation method described here keeps the isolated de-exined pollen alive and ready for further manipulation. Three nuclei could be visualized in the de-exined pollen, as indicated by DAPI fluorescence. Finally, the presence of cellulose, pectin, and callose indicated that the intines of the isolated de-exine pollen were intact.

Cytological studies
Cellulose, an essential component of the cell wall, was found to be present on the \(P. \) bungeana and \(P. \) wilsonii pollen intine. Pectins, the other main cell wall component of growing pollen tubes, are synthesized in the Golgi apparatus, methylesterified, modified with side chains, and subsequently released into the apoplastic space as highly methylesterified polymers (Micheli, 2001; Parre and Geitmann, 2005). Different kinds of pectins exist in plant cell walls, and the recycling of cell-wall pectins is essential for a proper assembly of the cell wall (Ridley et al., 2001; Šamaj et al., 2004). Acidic pectins (JIM5-reactive pectins) are biochemically produced from methylesterified pectins (JIM7-reactive pectins) (Baluška et al., 2002; Ridley et al., 2001). Esterified pectin contributes to the extensibility and elasticity of the cell wall, whereas unesterified pectin contributes to its rigidity during pollen germination and tube growth. In this study, both esterified and unesterified pectins were identified in the pollen intines of the two gymnosperm species, suggesting that they play an essential role in germination. Callose, a specialized wall material that is composed of \(\beta\)(1-3)-linked glucose polymers, and fluoresces brightly when stained with decolorized aniline blue, is synthesized in plants during differentiation processes and contributes to morphogenesis during reproduction (Peel et al., 1997; Tucker et al., 2001). Callose is observed in mature angiosperm pollen (Dumas and Knox, 1983). For example, in \(Populus\) pollen, callose is only present in the intines of a portion of mature pollen grains (Ashford and Knox, 1980). In contrast, in mature sunflower pollen, callose is present in the outermost wall layer (Vithanage and Knox, 1979). A thin callosic wall was present in \(Cucurbita pepo\) pollen (Nepi et al., 1995). Teng et al. (2005) reported that mature \(Leymus chinensis\) pollen grains contain almost no callose. A recent experiment in \(Arabidopsis\) documented that callose is essential for pollen wall patterning but not for tube growth (Nishikawa et al., 2005). In the
present study, callose was observed at a site corresponding to the sacci in *P. bungeana* pollen, but evenly present on the *P. wilsonii* intine surface. Therefore, the distribution of callose in the mature pollen wall may be species dependent.

The results obtained from immunolabeling with LM 2 antibodies, which recognize AGPs, showed that arabinogalactans, the main hemicellulose component that contributes to the mechanical properties of walls, were detected on the pollen intine in the plants used in this study. AGPs have important roles in various aspects of plant development, including the reproductive process, and have been implicated in transmembrane and cell–cell signaling pathways in a variety of plants (Pennell, 1998; Serpe and Nothnagel, 1999). Furthermore, AGPs have important roles during pollen germination and tube growth in *Actinidia deliciosa* (Abreu and Oliveira, 2004). Based on the results of the present study, AGPs on the pollen intine may take part in the above processes, as recognition molecules between the pollen and the stigma.

The presence of cellulose, pectin, and callose in pollen intines of the two species was revealed by fluorescence microscopy and further confirmed by FTIR analysis, implying that the isolated intines were complete. This result also agreed with that of Pacini et al. (1999). FTIR, a powerful, non-invasive technique for the quantitative assay of a variety of functional groups, is also widely used for the rapid evaluation of the chemical composition of plant cell walls (Chen et al., 1998; Coimbra et al., 1998; Mouille et al., 2003; Zeier and Schreiber, 1999), and is employed for the analysis of components of the pollen grain exine (Domínguez et al., 1999). In the present study, we analyzed the composition of the gymnosperm intine by the FTIR technique for the first time. The characteristics and content of pectin, the types of hemicelluloses, and the abundance of cellulose can be correlated with the rigidity, strength, and elasticity of the pollen intine. Two independent networks determine the mechanical properties of plant cells: the xyloglucan/cellulose mesh provides rigidity and strength parallel to the cell surface, and the more elastic pectin network permits more rapid adaptation to mechanical stress and alleviates stress gradients across the thickness of the wall.

Lectins play an essential role during plant defense responses (Peumans and Van Damme, 1995) and recognition processes, such as the symbiosis between *Rhizobium* and legumes (Hirsch, 1999). Lectin-binding sites on the pollen intine might function as recognition molecules or defense molecules when the pollen is released under stress conditions, as in this study.

The experimental system of de-exined pollen will be of practical applications. Through the comparison of intact pollen with de-exined pollen, the biological function of the exine can be studied and, by comparing de-exined pollen with pollen protoplasts, the biological function of the intine can be investigated. Furthermore, the system could strengthen knowledge of the functions of and relationships between the exine, the intine, and the protoplast in the processes of pollination and fertilization (Xia et al., 1996). De-exined pollen provides a powerful tool for evaluating marker-gene expression in *N. tabacum* pollen. Pollen without the exine covering should more easily accept foreign DNA for genetic transformation, as demonstrated in tobacco (Shi et al., 1996; Wang et al., 1998). Previous studies have shown that the *N. tabacum* exine is not absolutely necessary for pollen germination. However, the exine affects the germination rate in that the conditions required for the germination of de-exined pollen are more complex than those for intact pollen grains.

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