

A study on the allergenicity and ontogeny of *Acacia farnesiana* pollen grains in guinea pigs

Saeed Irian · Ahamad Majd ·
Aboozar Hoseinizadeh · Parisa Jonubi

Received: 11 October 2011 / Accepted: 9 April 2012
© Springer Science+Business Media B.V. 2012

Abstract Pollen grains as the angiosperm male gametophytes transfer male genetic material during sexual reproduction. Pollen grains are among the important plant allergens, such that almost 80–90 % of plant allergens are of pollen origin. *Acacia farnesiana* is a plant with economical values, and due to its resistance to dry climates, it has had a widespread distribution in Southern Iran. This study was aimed at investigating the allergenicity and the ontogeny of pollen grains of *A. farnesiana*. Pollen grains were collected from the blossoms and flowers of *A. farnesiana* in the suburbs of Ahvaz-Iran. Pollen extracts (15 %) were prepared in PBS (pH 7.2). 4–6-week-old male guinea pigs (*Hartly*) were used for allergenicity tests. Skin tests showed a significant increase in flare diameter. Clinical tests also showed a significant change in the levels of eosinophils, neutrophils, and IgE. Histotechnical analysis was performed on male gametophytes, and photographs were taken using a camera-equipped light microscope. Pollen characteristics were identified using both light and electron microscopy. Sporoderm ultrastructure and pollen morphology were studied using Scanning Electron Microscopy. PAGE analysis of the total

protein content of mature pollen grains showed seven clear bands of 10–83 kDa.

Keywords Pollen grain · Allergenicity ·
Acacia farnesiana · Ontogeny

1 Introduction

Acacias are among the important tree and shrub species growing in the shores of the Persian Gulf and the sea of Oman. Some species of the genus are natural elements of the region's flora, despite some other species are cultivated by people. Their unique root system allows water absorption from the lower levels of the ground. For this reason, they have been adapted to grow in dry climates. The flowering cycle normally starts early in the spring and continues through the midsummer. Different species of *Acacia* have been used for animal feed, woodworking, preparing flower extracts, pharmacology, and in the tools of farmers and fishers, in particular the boat industry.

Allergy to *Acacia* has been reported by several studies. Allergic rhinitis, also known as hay fever, and asthma are allergic reactions commonly observed in wood workers exposed to pollen from *Acacia* or *Acacia* wood (Wood-Baker and Markos 1997; De Zotti and Gubian 1996). Cross-reactivity between *Acacia* tree and Rye grass pollen allergens has also been described (Howlett et al. 1982). Date palm pollen (*Phoenix dactylifera*) has been shown to cross-react

S. Irian (✉) · A. Majd · A. Hoseinizadeh · P. Jonubi
Department of Biology, Tarbiat Moallem University,
Tehran, Iran
e-mail: irian@tmu.ac.ir

with antigens from Sydney golden wattle (*Acacia longifolia*) pollen (Kwaasi et al. 2002). Of the 306 allergic rhinitis patients who were evaluated for their skin prick test response to 27 common regional aeroallergens in Iran, *A. longifolia* pollen was positive in 29.3 % (Fereidounia et al. 2009). In a study of 327 adult patients, with diseases of suspected allergic origin, who were examined in a hospital in the United Arab Emirates, skin prick testing showed that among the 244 patients (74.6 %) with positive results, *Acacia* was positive in 25.6 % (Bener et al. 2002). In a study of aeroallergens in sandstorm dust investigated in Riyadh, Saudi Arabia, *Acacia* was among the most abundant aeroallergens (Kwaasi et al. 1998). In a different study in Saudi Arabia, of 1,159 patients tested for sensitization to inhalants, *Acacia* was positive in 29 % (Suliaman et al. 1997).

The aim of the present study was to further investigate the allergenicity of *A. farnesiana* pollen study, its developmental stages, and determine the total protein content.

2 Materials and methods

2.1 Allergenicity assays

2.1.1 Sampling

Acacia farnesiana (Fabales, Mimosaceae) pollens and flowers (very young blossoms to completely opened flowers) were collected from the suburbs of Ahvaz-Iran. Pollen grains were size-selected using a mesh with holes of 60 µm in diameter.

2.1.2 Animals and sensitization

Male 4–6-week-old guinea pigs (Hartly), weighting 350–500 g, were purchased from the Pasture Institute of Iran and kept in a room at 25 °C with an 8-h light/8-h dark condition. They were fed a standard laboratory diet and given water with added vitamins. These animals were then divided into two groups of seven animals each and sensitized with 100 µl *A. farnesiana* pollen extract (containing 50 µg protein in phosphate-buffered saline) with 50 µg Al(OH)₃ added as an adjuvant. Al(OH)₃ was suspended in saline buffer and added to pollen extract. Treatments were repeated six times, for 3 weeks, twice a week.

2.1.3 Skin test

Acacia farnesiana pollen extract was prepared by the method of Sheldon et al. (1967), and skin test was performed on guinea pigs. Each animal was tested with 100 µl of the total extract diluted with 0.05 M phosphate-buffered saline (pH 7.4) containing 50 µg protein without adjuvant. The negative control received 100 µl of buffered saline. Skin reactions were read 12 h from the beginning of the test and quantified on the basis of flare diameter.

2.1.4 Clinical and serological tests

Prior to each experiment, the guinea pigs were bled once. Blood samples were drawn directly from the hearts (Aberg 1989). After treatment with pollen extract, the guinea pigs were anesthetized with chloroform. Blood was then obtained directly from the hearts. Sera from all samples were stored at –70 °C until used. The sera from seven *A. farnesiana* sensitized animals were individually tested for IgE reaction to *A. farnesiana* pollen grains. Sera from seven animals that were not sensitized to *A. farnesiana* pollen were also tested as control sera. Serum total IgE level was determined by ELISA, using an IgE guinea pig elisa kit (Cusabio Co.) (Mondal et al. 1997). The serum IgE level was expressed in nanograms per milliliter. Blood smears of healthy controls were compared with those of the experimental animals. Total numbers of different kind of blood cells, specially, eosinophils and neutrophils, were determined in the two groups and statistically analyzed.

2.2 Microscopy

2.2.1 Light microscopy

Flowers and blossoms were fixed in FAA (37 % formaldehyde, glacial acetic acid, and 96 % ethanol in a 2:0.5:17 ml ratio), washed with ddH₂O, and dehydrated using ethanol solutions of increasing concentrations. Following the replacement of ethanol with toluene, samples were saturated with melted paraffin. Slices of 8–10 mm thin were prepared and stained with Hematoxylin–Eosin before taking images under a Zeiss light microscope.

To study pollen wall structures, *Acacia* pollen grains were subjected to acetolysis analysis using the

method of Erdtman (1960) before being examined under a light microscope (Zeiss).

2.2.2 Scanning electron microscopy

For ultrastructural analysis, *A. farnesiana* pollen grains were studied by scanning electron microscopy. Purified pollen grains were fixed with glutaraldehyde, postfixed with osmium, dehydrated, and dried prior to gold coating. Coated samples were then analyzed using a scanning electron microscope (XL30, Philips, Holland).

2.3 Protein studies

Extraction of pollen protein from *A. farnesiana* pollen grains was carried out separately at 4 °C in Tris–HCl buffer, pH 7.6. A 12 % SDS polyacrylamide gel electrophoresis was performed for the total soluble protein according to the method of Laemmli (1970). Soluble proteins were extracted in sample buffer (0.125 M Tris–HCl (pH 6.8); 4 % SDS; 20 % glycerol; 10 % β -mercaptoethanol; 0.1 % BPB) with heating for 3–4 min at 100 °C before loading. The amount of protein was 10 μ g per channel, and the total current applied was 14 mA. The gel was run in Tris–glycine buffer (pH 8.3) with 0.1 % SDS and calibrated with a marker protein obtained from Sigma. Protein concentration of the pollen extract was determined by the method of Bradford (1976).

2.4 Statistical analysis

Comparisons were analyzed using the One-way ANOVA, and significant differences between groups were analyzed by Duncan's multiple-range test. P value <0.05 was considered significant.

3 Results

3.1 Allergenicity

Allergenicity of *A. farnesiana* pollen grains was assayed by skin, clinical, and serological tests. The average diameter of the flare caused by the mature

pollen and control lines were compared. The maximum sensitivity to the allergenic mature pollen grains included a flare with a 3.9 cm in diameter, which is significantly ($P < 0.0001$) different than that of the control animals (Fig. 1a).

Counts of eosinophils and neutrophils, and the IgE levels, also showed a significant ($P < 0.001$) difference with those of the control line (Fig. 1b–d).

3.2 Structural and ultrastructural analysis

Figure 2 shows the cross-section of a young anther at an early stage of development. The three stainable sections include: the epidermis (E), the protoderm (PD), and a dividing ground meristem (GM). As shown in Fig. 3, the GM layer gives rise to an endothecium layer (EL), a middle layer (ML), and a Tapetum layer (T), while the central cell mass gives rise to multilateral pollen mother cells (Pmc) with massive nuclei. These cells are surrounded by the tapetum layer. The tapetal cells contain a highly stainable nucleus that is separated from the middle layer during anther development (Fig. 4).

Having gone through meiosis 1 and 2, pollen mother cells are shown to have produced tetrads (meiospores) of haploid cells, surrounded by a special wall (SW) of callosic nature (Fig. 5a). At this stage, the tapetal cells are vascularized, and their dimensions expanded (Fig. 5b). Figure 5c shows meiospores whose callose walls have been eradicated, and each meiospore is now connected to the neighboring meiospore through cytoplasmic bridges and remain interconnected through endoeuxinic bridges in polyad throughout their development.

The tapetum layer starts disintegrating, and by doing so, the exine and intine layers form on the pollen grains, while the endoeuxinic bridges keep the monads together in polyad (Fig. 6). Mitotic division of the young uninuclear microspores gives rise to binuclear microspores (Fig. 7). Following the formation of generative and vegetative nuclei, the former is pushed to the side away from the latter, and a pectocellulosic wall forms between them, eventually leading to the formation of mature pollens.

Acetolysis analysis showed that *A. farnesiana* pollen grains are spherical ovoidal with openings at the proximal surface and colpus at the distal surface

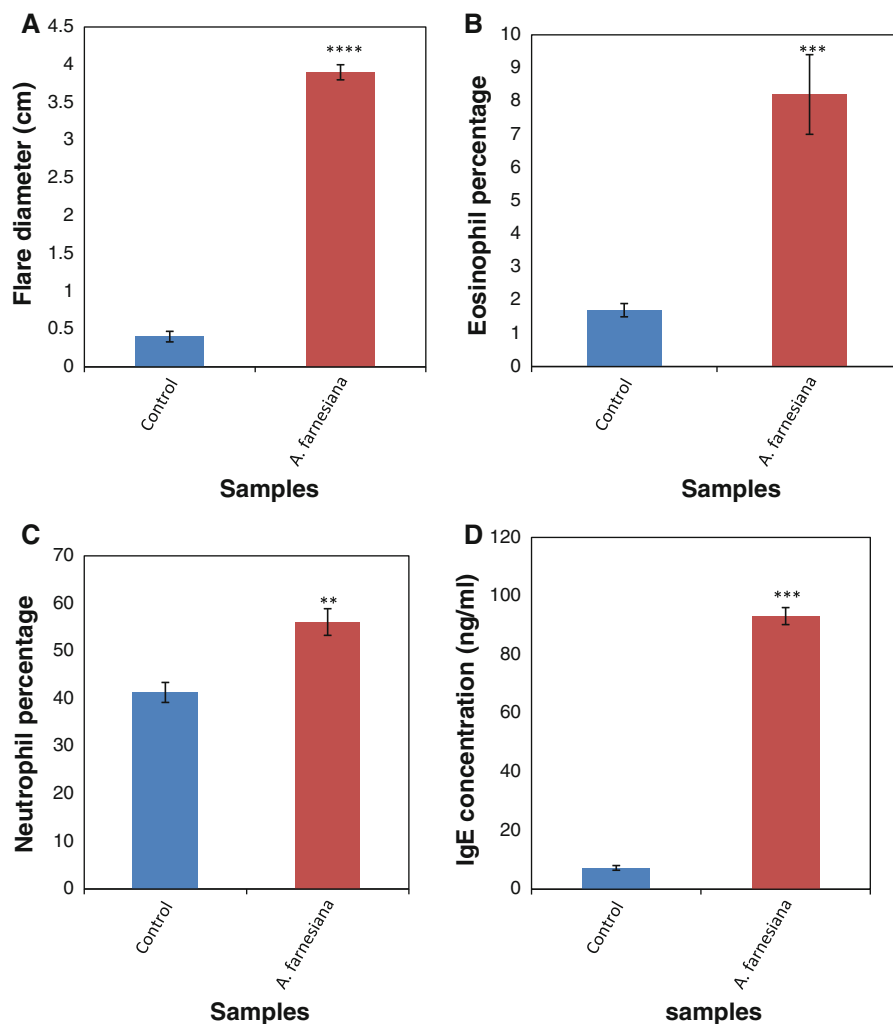


Fig. 1 **a** Pollen extract skin test showing flare diameter. **b** Serological data for the percentage of eosinophils. **c** Serological data for the percentage of neutrophils. **d** Serological data for

blood IgE levels. Samples: control: animals injected with PBS buffer; *A. farnesiana*: animals injected with *A. farnesiana* pollen extract in PBS buffer. *** $P < 0.001$, **** $P < 0.0001$

with a diameter of about 50–60 μm , scattered as an interconnected polyad (Fig. 8a). Images from the SEM showed an *A. farnesiana* pollen grain with an uneven, porous and wrinkled exine (Fig. 8b, c).

3.3 PAGE analysis

Bradford assay performed on total protein, extracted from *A. farnesiana* pollen grains, showed a 6.3 mg/g protein concentration. 12 % SDS-PAGE analysis revealed several bands in the range of 10–83 kDa. These included 2 bands of 16 and 22 kDa, a major band of 27 kDa, and 4 bands of 32, 50, 66, and 83 kDa (Fig. 9).

4 Discussion

4.1 *A. farnesiana* pollen allergenicity

In the past few decades, the reported cases of the allergic rhinitis and other related diseases like asthma and urticaria have grown at tremendous rate as recorded from various epidemiological studies carried out all over the world (Björkstén et al. 2008). The pollen grains constitute one of the important components of air and are considered as major causative agent of respiratory disease, as is evident by the spurt of literature from across the world during last few decades (Clot 2003; Fang et al. 2001).

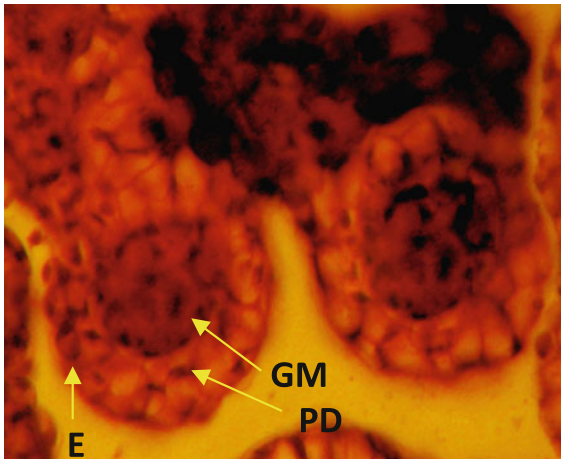


Fig. 2 Cross-section of a young anther: epidermis (E); protoderm (PD); ground meristem (GM); mag. $\times 100$

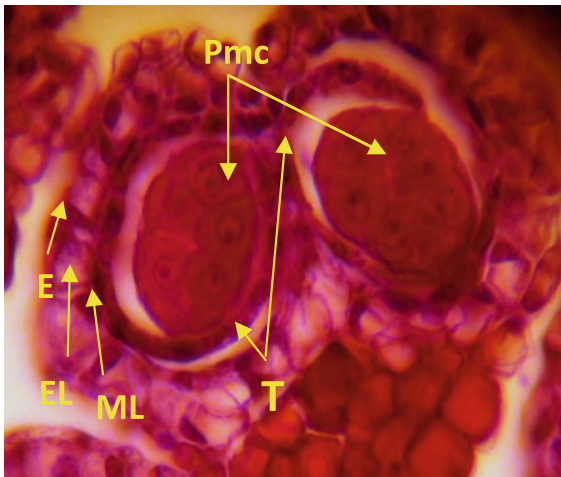


Fig. 3 Cross-section of the anther: epidermis (E); endothecium layer (EL); middle layer (ML); tapetal layer (T); pollen mother cell (Pmc); mag. $\times 100$

Several recent studies have shown that *Acacia* pollens constitute some of the major sensitizing aeroallergens in the Persian Gulf region (Fereidounia et al. 2009; Bener et al. 2002; Kwaasi et al. 1998; Suliaman et al. 1997). In the present study, based on the results of our skin and clinical tests performed on guinea pigs for the allergenicity of pollen grains, it appears that *A. farnesiana* pollen grains are allergenic. These included: a strong reaction following the skin test; a rise in blood eosinophil levels; presence of eosinophils in eye and nasal secretions (Rothenberg 2007); and a rise in blood IgE levels (Majd et al. 2004;

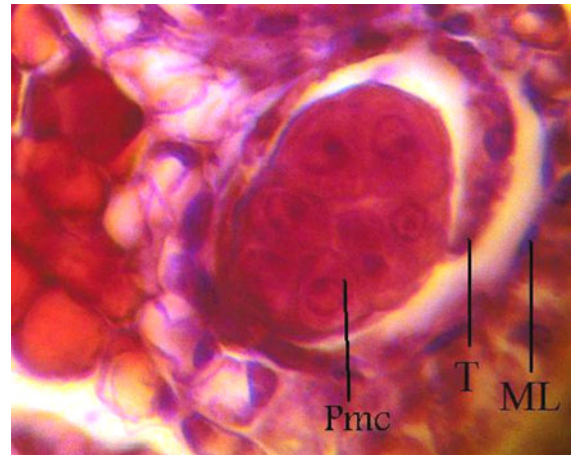


Fig. 4 Cross-section of an anther showing the tapetal cells (T) with highly stainable nuclei and separated from the middle layer (ML). Pmc pollen mother cell, mag. $\times 400$

Onishi et al. 2007), all of which are indicative of the allergenicity of *A. farnesiana* pollen grains.

4.2 *A. farnesiana* pollen development

Analysis of the developmental stages of *A. farnesiana* anther and pollen grains indicated the presence of a protodermal cell layer and a central cell mass, both with a high level of stainability. Boke (1949) pointed out the presence of a protoderm and a ground meristem cell mass at an early stage of anther formation

Cells of the ground meristem give rise to the cells of anther wall and spore mother cells. The middle layer includes two cell layers of both temporary and unstable in nature that are disintegrated during anther development (separation of tetrads). This study showed the temporary nature of this layer in *A. farnesiana*, a finding that is in line with those of others (e.g. Busse et al. 1969).

The tapetum layer of *A. farnesiana* is of a secreting type, which is disintegrated at the final stages of pollen development, following the formation of the young microspores. The remnant of the disintegrated tapetum layer is then absorbed by the microspores, and thus is undetectable during the formation of mature pollen. The increase in the growth rate of tapetum layer during anther development through tetrad formation, and the increase in the density of the growing microspores that are in contact with this layer, along with its gradual disintegration during the growth of microspores, are

Fig. 5 Cross-section of an anther showing the tetrad stage (**a–c**): nucleus (*N*); special wall (*SW*); tapetum layer (*T*); haploid cell (*HC*). Mag. $\times 400$

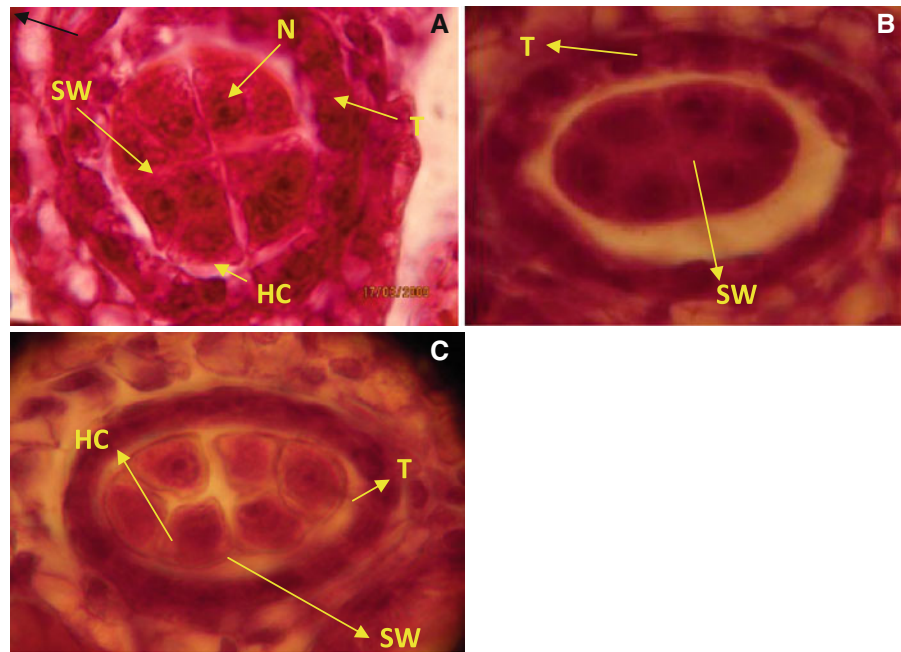


Fig. 6 Tapetal cell (*T*) disintegration, precipitation of wall debris, and formation of the pectocellulosic wall (*PW*). Mag. $\times 400$

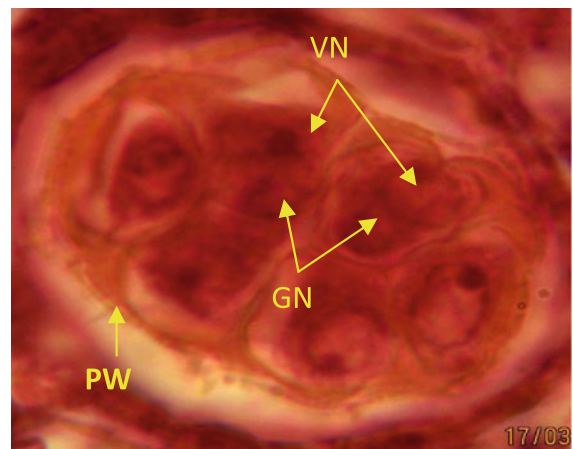


Fig. 7 Generative cell surrounded by the vegetative cell cytoplasm. Vegetative nucleus (*VN*); generative nucleus (*GN*); pollen wall (*PW*). Mag. $\times 600$

all indicative of a nutritional role for this layer (Dickinson et al. 2000).

During anther development, the primary pollen mother cells are differentiated from the ground meristem, quickly expand in volume, and take on a multilateral appearance with a thin pectocellulosic wall surrounding a nucleus that takes up a great volume of the cell. The nucleus normally contains a big nucleolus, which makes these cells distinguishable

from those that give rise to cells of the anther wall (Dickinson et al. 2000).

In the beginning of prophase I, each of the pollen mother cells secretes a layer of a special wall. This wall, which is basically callosic in nature, also contains lipids and polysaccharides and remains intact until the formation of tetrads. This wall prevents the dividing cells interacting with other cells. Formation and stability of this wall in *A. farnesiana* is in line with findings in members of the angiosperms by others

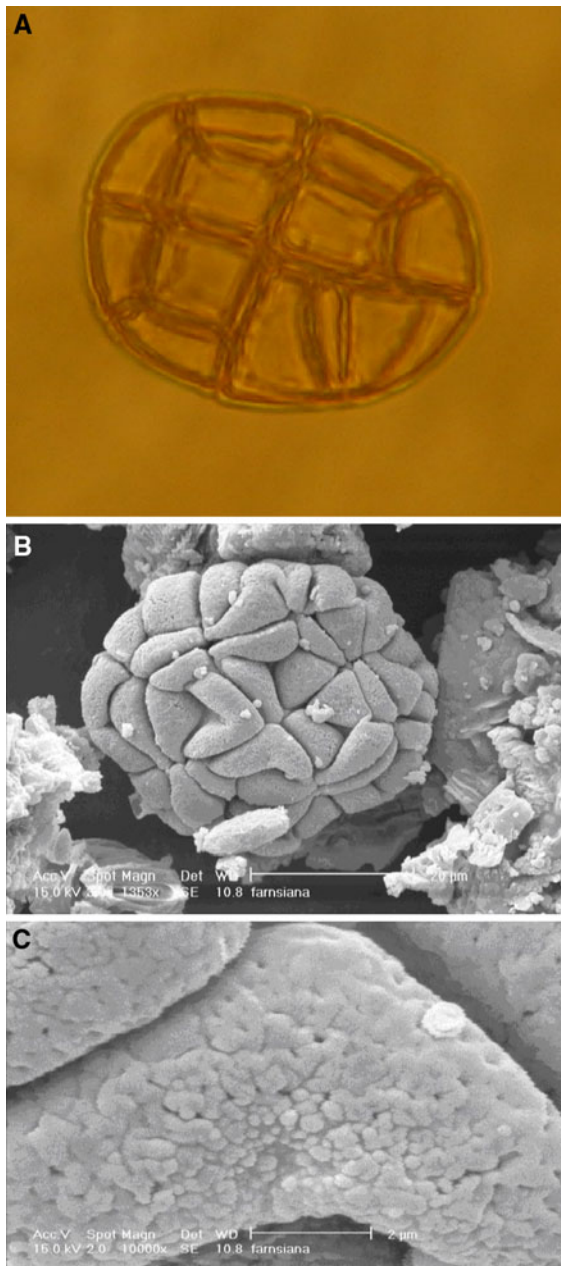


Fig. 8 **a** Light microscopy image of the acetolyzed *A. farnesiana* pollen grain (mag. $\times 100$). **b** SEM showing *A. farnesiana* pollen grains. **c** SEM showing decorations on the exine layer of *A. farnesiana* pollen grain

(Bedinger 1992). At the end of meiosis II, tetraspore separation in *A. farnesiana* as in other dicots is quadrilateral, and division of the cytoplasm that results in the separation of tetrads is simultaneous, possibly as a consequence of the cytoplasmic connections



Fig. 9 Image of the stained SDS-PAGE gel showing bands of the total protein extracted from *A. farnesiana* pollen grain (right) and the molecular marker (left) (sizes are in kDa)

between the spore mother cells (Bedinger 1992). Following meiosis, and during the disintegration of callose wall, precipitation of the primary wall results in the monads of the polyad pollen grains to stick together. In polyad pollen grains, the cytoplasmic channels keep neighboring microspores connected. These cytoplasmic channels would remain intact until the end of meiosis and sometimes even until the complete disintegration of the callose wall (Knox and McConchie 1986). Therefore, it is likely that polyad formation takes place following meiosis. Following the maturation of young microspores in angiosperms, a stage of mass increase, and vacuole formation follows, which results in a volume increase in microspores. Based on the morphological estimation of polyad size throughout development, it became apparent that vacuole formation and the fast growth rate of polyads do not take place in *A. farnesiana* (Evans et al. 1992). Following the formation of the wall, a change in the cytoplasmic content of microspores occurs, which includes the formation of small

vesicles in the cell. Following this change, tapetal cells disintegrate, and the resulting remnants move toward the polyad, a possible sign of microspores preparing to undergo mitosis, which has also been shown through TEM analysis (Melissa et al. 1993).

Structural and ultrastructural analyses of *A. farnesiana* pollen grains using both light and scanning electron microscopy revealed the existence on the exine layer of a pattern of striation, openings, pores and decorations, and it became clear that the exine surface is porous with a deep striation pattern, giving the pollen surface a wrinkled appearance with a central cavity, wherein both antigenic and allergenic materials could accumulate (Heslop-Harrison 1966). The existence of such decorations on pollen grains of the family Mimosaceae and the Acacia genus has also been reported by others (Caccavari 2002; Tantawy et al. 2005).

4.3 *A. farnesiana* pollen protein analysis

Electrophoretic (SDS-PAGE) analysis of *A. farnesiana* total pollen protein content revealed several different bands in the range of 10–83 kDa. Howlett et al. (1982) reported on the cross-reactivity between *Acacia* species and *Lolium perenne* (Ryegrass) pollens, which might be due to the presence of similar proteins in different pollen extracts. Ryegrass serves as an important source of seasonal allergens in hot and temperate climates. This plant contains 17 allergens with MWs ranging from 11 to 86 kDa with a strong reactivity toward IgE. Allergens LoLp1 with a MW ranging from 28–35 kDa (Singh et al. 1990) and LoLp5 with a MW of 31 kDa are among its major allergens (Singh et al. 1991). Since *A. farnesiana* pollen grains showed a strong allergenicity, and the band with the highest density corresponded to the 27 kDa protein, it appears likely that the 27 kDa polypeptide, detected in the present study, represents a strong candidate as one of several factors responsible for the allergenicity of *A. farnesiana* pollen grains. This may further be tested through immunoblot analysis.

5 Conclusion

The present investigation used different tests, performed on guinea pigs, to demonstrate the allergenicity of *A. farnesiana* pollen grains. In addition, the morphological changes of both the anther tissue and

the pollen cells at different stages of the development, as well as the total protein content of the mature pollen, were revealed.

References

- Aberg, N. (1989). Asthma and allergic rhinitis in Swedish conscripts. *Clinical and Experimental Allergy*, 19, 59–63.
- Bedinger, P. (1992). The remarkable biology of pollen. *The Plant Cell*, 4, 879–887.
- Bener, A., Safa, W., Abdulhalik, S., & Lestringant, G. G. (2002). An analysis of skin prick test reactions in asthmatics in a hot climate and desert environment. *Allergologia et immunopathologia (Paris)*, 34(8), 281–286.
- Björkstén, B., Clayton, T., Ellwood, P., Stewart, A., Strachan, D., & ISAAC Phase III Study Group. (2008). Worldwide time trends for symptoms of rhinitis and conjunctivitis: Phase III of the International Study of Asthma and Allergies in Childhood. *Pediatric Allergy and Immunology*, 19(2), 110–124.
- Boke, N. H. (1949). Development of the stamens and carpels in *Vinca rosea* L. *American Journal of Botany*, 36, 535–547.
- Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248–254.
- Busse, P. A., Galen, D. F., & Lersten, N. R. (1969). Pollen and tapetum development in *Desmodium glutinosum* and *D. Illinoense* (Papilionoideae; Leguminosae). *American Journal of Botany*, 56, 1203–1208.
- Caccavari, M. A. (2002). Pollen morphology and structure of tropical and subtropical American genera of the *Piptadenia*-group (Leguminosae: Mimosoideae). *Grana*, 41, 130–141.
- Clot, B. (2003). Trends in airborne Pollen: An overview of 21 years of data in Neuchâtel (Switzerland). *Aerobiologia*, 19(3–4), 227–234.
- De Zotti, R., & Gubian, F. (1996). Asthma and rhinitis in wooding workers. *Allergy and Asthma Proceedings*, 17(4), 199–203.
- Dikinson, H. G., Elleman, C. J., & Doughty, J. (2000). Pollen coating-chimaeric genetic and new function. *Sexual Plant Reproduction*, 12, 302–309.
- Erdtman G. (1960). The acetolysis method. A revised description. *Svensk Botanisk Tidskrift* 54, 556–564.
- Evans, D. E., Taylor, P. E., Singh, M. B., & Knox, R. B. (1992). The interrelationship between the accumulation of lipids, protein and the level of acyl carrier protein during the development of *Brassica napus* pollen. *Planta*, 186, 343–354.
- Fang, R., Xie, S., & Wei, F. (2001). Pollen survey and clinical research in Yunnan, China. *Aerobiologia*, 17(2), 165–169.
- Fereidounia, M., Farid Hossini, R., Jabbari Azada, F., Ali As-sarezadegana, M. A., & Varasteh, A. (2009). Skin prick test reactivity to common aeroallergens among allergic rhinitis patients in Iran. *Allergologia et immunopathologia*, 37(2), 73–79.
- Heslop-Harrison, J. (1966). Cytoplasmic continuities during spore formation in flowering plants. *Endeavour*, 25, 65–72.

- Howlett, B. J., Hill, D. J., & Knox, R. B. (1982). Cross-reactivity between *Acacia* (wattle) and rye grass pollen allergens. Detection of allergens in *Acacia* (wattle) pollen. *Clinical and Experimental Allergy*, 12(3), 259–268.
- Knox, R. B., & McConchie, C. A. (1986). Structure and functions of compound pollen. In S. Blackmore & I. K. Ferguson (Eds.), *Pollen and spores: Form and function*. London: Academic press.
- Kwaasi, A. A., Harfi, H. A., Parhar, R. S., Saleh, S., Collison, K. S., Panzani, R. C., et al. (2002). Cross-reactivities between date palm (*Phoenix dactylifera* L.) polypeptides and foods implicated in the oral allergy syndrome. *Allergy*, 57(6), 508–518.
- Kwaasi, A. A., Parhar, R. S., al-Mohanna, F. A., Harfi, H. A., Collison, K. S., & al-Sedairy, S. T. (1998). Aeroallergens and viable microbes in sandstorm dust. Potential triggers of allergic and nonallergic respiratory ailments. *Allergy*, 53(3), 255–265.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680–685.
- Majd, A., Chehregani, A., Moin, M., Gholami, M., Kohno, S., Nabe, T., et al. (2004). The effects of air pollution on structures, proteins and allergenicity of pollen grains. *Aerobiologia*, 20, 111–118.
- Melissa, A., Fitzgerald, D., Calder, M., & Knox, R. B. (1993). Character states of development and initiation of cohesion between compound pollen grains of *Acacia paradoxa*. *Annals of Botany*, 71, 51–59.
- Mondal, A. K., Parui, S., Biswas, S. R., & Mandal, S. (1997). Identification of the allergenic proteins of *Ipomoea fistulosa* pollen: Partial characterized and sensitivity test. *Grana*, 36, 301–305.
- Onishi, N., Kawamoto, S., Ueda, K., Yamanaka, Y., Katayama, A., Suzuki, H., et al. (2007). Dietary pulverized konjac glucomannan prevents the development of allergic rhinitis-like symptoms and IgE response in mice. *Bioscience Biotechnology and Biochemistry*, 71, 2551–2556.
- Rothenberg, M. E. (2007). Eosinophils in the new millennium. *Journal of Allergy and Clinical Immunology*, 119, 1321–1322.
- Sheldon J. M., Lovell R. G., Mathews K. P. (1967). *A manual of clinical allergy*. London: W. B. Saunders Co. Phil. Cited by Shivpuri C. R., Shivpuri D. N. Re-evaluation of intradermal tests. *Asp of All and Appl Immunol* 9, 205–214.
- Singh, M. B., Hough, T., Theerakulpisut, P., Avjioglu, A., Davies, S., Smith, P. M., et al. (1991). Isolation of cDNA encoding a newly identified major allergenic protein of rye-grass pollen: Intracellular targeting to the amyloplast. *Proceedings of National Academy of Sciences of USA*, 88, 1384–1388.
- Singh, M. B., Smith, P. M., & Knox, R. B. (1990). Molecular biology of rye-grass pollen allergens. *Monographs in Allergy*, 28, 101–120.
- Suliaman, F. A., Holmes, W. F., Kwick, S., Khouri, F., & Ratard, R. (1997). Pattern of immediate type hypersensitivity reactions in the Eastern Province, Saudi Arabia. *Annals of Allergy Asthma and Immunology*, 78(4), 415–418.
- Tantawy, M. E., Khalifa, S. F., Hamed, K. A., & Elazab, H. M. (2005). Palynological study on some taxa of Mimosoideae (Leguminosae). *International Journal of Agriculture and Biology*, 6, 857–868.
- Wood-Baker, R., & Markos, J. (1997). Occupational asthma due to blackwood (*Acacia Melanoxylon*). *Australian and New Zealand Journal of Medicine*, 27(4), 452–453.