Journal of BIOLOGICAL RESEARCHES
ISSN: 08526834 | E-ISSN:2337-389X
Volume 24| No. 1| December| 2018

Original Article

The orbicules and allergenic protein of African tulip tree (*Spathodea campanulata* P.Beauv.): a roadside ornamental plant in Malang, Indonesia

Janis Damaiyani[1*], Yekti Asih Purwestri[2], Issirep Sumardi[3]
[1]Purwodadi Botanic Garden, Indonesian Institute of Sciences, Indonesia
[2,3]Biology Faculty, Gadjah Mada University, Yogyakarta, Indonesia

Abstract

Orbicules or ubisch bodies are capable carrying specific pollen antigens which responsible for presenting allergenic activity in the atmosphere. Currently, there is no report on the orbicules and allergenic protein in African tulip tree. Thus, this research was aimed to investigate the presence of orbicules and allergenic protein in African tulip tree and to determine their potential as allergen source. The ultrastructure of orbicules were detected using Transmission Electron Microscope (TEM) and Scanning Electron Microscopy (SEM). Allergenic proteins were analyzed based on the molecular weight using SDS-PAGE. The result showed that he orbicules were present in the tapetal cells. Furthermore, the pro-orbicules were released by tapetal cells and fill the loculus before anthesis. The SDS-PAGE analysis showing several proteins with a molecular weight of 10-70 kD were detected in African tulip tree and cauld be potential as source for allergen. Protein bands with a molecular weight 55 kDa, 48 kDa, 39 kDa, and 27 kDa appear with the darkest, clearest and thickest expression of genes compared to other bands. In other species, the protein bands with molecular weight 55 kDa, 48 kDa and 27 kDa were detected as allergen. This results indicated African tulip tree could be potential for pollen allergen from ultrastructure and allergenic protein observation.

Keywords: African tulip tree, allergenic protein, orbicules, pollen.

Received: 30 July 2018 Revised: 09 November 2018 Accepted: 19 November 2018

Introduction

Human allergic reactions can be caused by many environmental substances. Pollen is an important trigger of allergic reactions (Gunawan et al., 2008). Therefore, the selection of plants for roadside ornamental and public spaces should consider the allergic issue. African tulip tree is one of the roadside ornamental plants (Kirmanto, 2012), which are commonly found in Malang city (East Java, Indonesia). Its highly visible bunch of colorful flowers makes this plant as an attractive roadside ornamental plant (Chin, 2003). This taxa is cultivated in parks and recreation areas around the city. As the plant is used in public areas, it is important to investigate its safety, especially the allergenicity of its pollen.

Allergies to the pollen of flowering plant species significantly impacts on the health of people in many parts of the word. The allergic response causes hayfever or pollinosis (de Weerd et al., 2002). Pollen allergy is caused by proteins, glycoprotein (Zeb et al., 2017). Allergenic protein has molecular weight range between 10-70 kDa (Vigh-Conrad et al., 2010; Rengganis et al., 2008). Pollens contain many proteins, but only a relatively small number of these cause allergenic properties. Allergenic proteins are characterized solely on their ability to induce an allergenic response in sensitive individuals, no common structural or functional features of these molecules have been identified to explain this ability (de Weerd et al., 2002).

* Corresponding Author:
Janis Damaiyani
Purwodadi Botanic Garden – Indonesian Institute of Sciences, Jl Surabaya-Malang Km 65, Purwodadi, Pasuruan, East Java, Indonesia
Phone: +6282243585101 Fax: -
e-mail: j.damaiyani@gmail.com

Several authors have suggested that pollen-allergenic particles originating from plant parts other than pollen can occur in the atmosphere. Parts from inflorescences and/or leaves as well as the so-called orbicules or ubisch bodies are capable carrying specific pollen antigens responsible for the presence of allergenic activity in the atmosphere (Vinckier et al., 2005; D'Amato et al., 2007; Dinis et al., 2007; Ebi et al., 2009). Orbicules of *Corylus avellana* is very effective vectors of allergens after the pollen season (Vinckier et al., 2005). Similar study has also been reported on orbicules in *Betula pendula* also possible role in allergy. The orbicules of *B. pendula* are 2-4 pm in diameter, they can pass through the bronchiole of the lungs and cause bronchial asthma (El-Ghazaly et al., 1995).

Methods

Plant Material

Samples in this research were African tulip tree flowers that grow in Malang City (East Java, Indonesia) and used as road side plants. Collection and observation were performed from June 2013 - June 2014.

Ultrastructure Analysis

Detection of the Ubisch bodies was performed by analyzing the ultrastructure of anther cross section at the tetrad stage using Transmission Electron Microscope (TEM). The ultrastructure of anther inner wall of the at the tetrad stage and pollen solitary stage were analyzed by Scanning Electron Microscope (SEM).

The procedure outlined of TEM analysis was described by Bozzola & Russell (1998). Another samples at the tetrad stage were fixed in 2.5% (w/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 24 h, 4 °C. The samples were rinsed with the same buffer three times for 15 mins. Then the samples were soaked in 2% osmium tetraoksida and 2.5% $K_3Fe(CN)_6$ in the same

buffer for 2 h, 4 °C. The samples were rinsed again with the same buffer three times for 15 mins and dehydrated by passing through a grade series of ethanol (30%, 50%, 70%, 95%) for 15 mins. This was followed by infiltration, embedding, cutting and colouring by triple lead. Then the samples were examined under a Transmission Electron Microsscope (TEM) 'JEM 83 1010/ JEOL Electrone Microscope'.

The procedure outlined of SEM analysis was described by Pusposendjojo (1982). Sample of the anthers at the tetrad stage and pollen solitary stage were mounted in the holder by carbon tip. Then, the samples were coated with gold by sputter coater for 30 mins. Then the samples were examined under a Scanning Electron Microsscope (SEM) 'FEI tipe Inspects 25'.

Protein Extraction

Proteins of African tulip tree pollen from two kinds of development stage (anthesis and before anthesis) were extracted. The procedure outlined of protein extraction from pollen was described by Rengganis et al. (2008) with modification. Pollen grains were collected in a 1.5 mL microtube and soaked in liquid nitrogen overnight. This step was done to minimize proteolysis (Vanderplanck et al., 2014). The next day, the weight of samples were measured. Then they were pounded in a mortar. This step was done to break the cells. To separate the proteins from the other macromolecule such as lipids, the samples were suspended in 10% (w/v) ethanol absolute and were shaken using vortex for 30 mins at room temperature. The suspensions were centrifuged at 14000 rpm for 20 mins at 4 °C to avoid proteins denaturation. Supernatant containing ethanol with dissolved lipids were discarded and the precipitant was dried over tissue to vaporize the ethanol. The precipitant was then suspended in 5% (w/v) 0.5 M NaCl using vortex for 30 mins at room temperature to dissolve the proteins. The extract of proteins were included in the microtube and the protein contents were measured by the Bradford method with Bovine Serum Albumin (BSA) as the standard (Bradford, 1976). The extract of proteins were stored at -20 °C for further used.

Protein Profiling

The molecular weigth of proteins were measured by One-Dimentional Electrophoresis (SDS-PAGE). The procedure outlined of SDS-PAGE was described by Laemmi (1970). All buffers for SDS-PAGE were prepared according to Fatchiyah et al. (2011). Polyacrylamide gel was composed from 12.5% for separating gel and 4% for stacking gel. Each samples that contained 10 µg/mL of protein was heated at 100 °C for 5 mins and then incubated in a freezer for 10 min to denature the proteins. After cooling, the samples were equally mixed with buffer samples (10% SDS, 50% glycerol, 2-mercaptoethanol, 1% bromophenol blue, 1 M Tris-HCl pH 6.8), then were loaded in the wells. The gel was calibrated with the protein marker (Thermo Scientific PageRuler Prestained Protein Ladder) with molecular weights of 15, 25,35, 40, 55, 70, 100, 130, 147 and 170 kDa.

Electrophoresis was carried out at 25 mA, 100 Volt, for 80 mins. Gel was stained overnight by using Coomassie Briliant Blue staining. Subsequently gel was destained in the destaining solution (methanol, glacial acetic acid, and aquades) until the protein bands appeared clear. After destaining, the gel was photographed and preserved.

Results

Ultrastructure observation with TEM showed that the orbicules were present in the tapetal cells. They were lined on the surface of tapetal cells inner side (Fig. 1, white arrow). In this stage, the orbicules were called pro-orbicules. In the cytoplasm of tapetal cells, pro-orbicules appear as a grayish body lipid, named as gray bodies. The form of pro-orbicules were globular with a very small size and a diameter less than 1 µm.

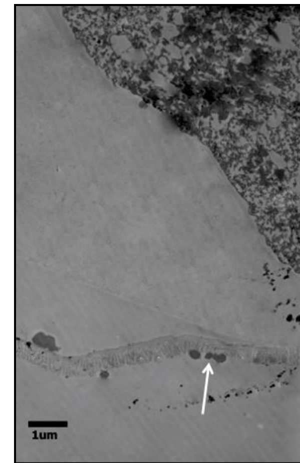

Figure 1. Electron micrographs of pro-orbicules (white arrow) lined on the surface of tapetal cells of African tulip tree

The observation of the anther before anthesis by SEM showed that the pro-orbicules were released by tapetal cells and fill the loculus (Fig. 2a, white arrow). Sporophytic protein and pro-orbicules were transferred directly to the exine. Some orbicules adhered with the exine layer (Fig. 2b, white arrow).

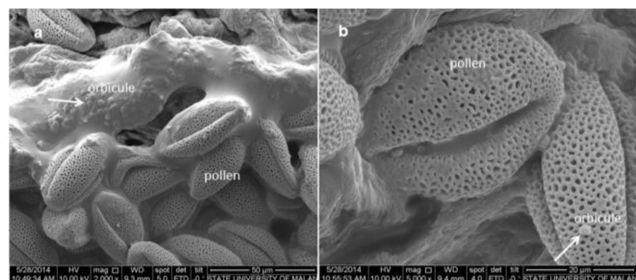

Figure 2. Electron micrographs of the orbicules in the tapetal cells of African tulip tree flowers before anthesis. [a] The orbicules filled the anther loculus (white arrow). [b] Some orbicules adhered with the exine layer (white arrow).

After anthesis, the orbicules were abundant, and densely packed at the inner surface of the tapetum (Fig. 3a, white arrow). The surface of the orbicules were smooth and have no ornament. Their shape irregularly and varies size. Generally, their size were less than 5 µm in diameter (Fig. 3b). The presence of orbicules both on

the surface of the innermost wall of the anther and the wall of pollen showed the species potential as allergen.

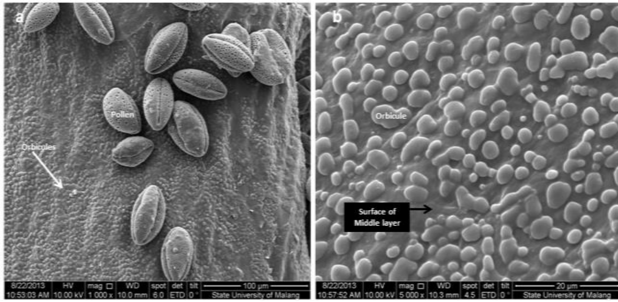

Figure 3. Electron micrographs of the orbicules which scattered on the surface of the innermost wall of the anther. [a] The orbicules filled and scattered the middle layer surface (white arrow). [b] Extended magnification show smooth orbicules surface and have no ornament.

Related to morphological characters, the potential of pollen alergenicity from African tulip tree was also supported by protein analysis. In this study, several proteins with a molecular weight of 10-70 kD were detected in both samples (before and after anthesis) (Figure 4). Figure 4 showe that each sample had 4 pieces of protein bands with a molecular weight of 55, 48, 39, and 29 kDa gene expressions that are thick, very clear, and appear dark compared to other protein bands. The intensity of protein expression decrease after anthesis than before anthesis.

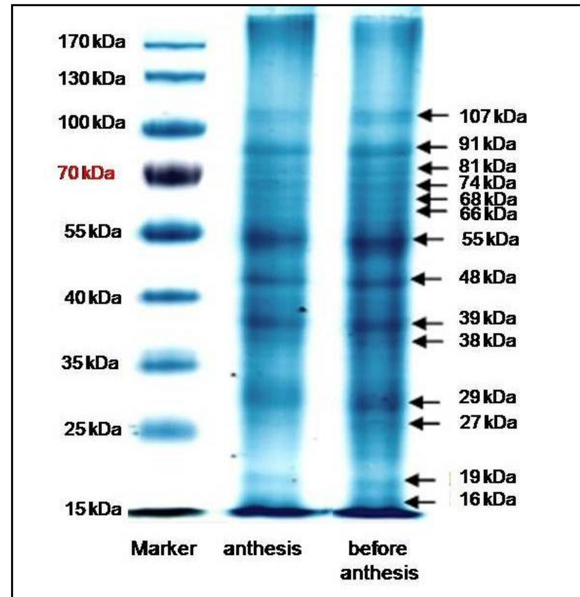

Figure 4. Coomassie Briliant Blue stainning of One-Dimentional Electrophoresis (SDS-PAGE) of African tulip tree pollen at different stages; anthesis and before anthesis.

Discussion

The role of pollen grains in allergic diseases has been reported in a number of studies. Previously, it is generally believed that most of the allergic reaction in human beings was caused by anemophilous species (Behrendt & Becker, 2001; Prakashkumar et al., 1998). However, other studies showed animal pollinated species such as *Dolichandrone platyclyc* also caused allergy by 16.1% (Mrindula et al., 2011). de Weerd et al. (2002) also stated that entomophilic species such as *Pyrus pyrifolia* (Japanese pear) causes allergic for farmers. African tulip tree is known as one of the anemophilous species, thus this tree could be potential as source for pollen allergen.

Observations of the anther showed orbicules on African tulip trees. Vinckier & Smets (2001) investigate 15 allergenic species from Betulaceae, Chenopodiaceae, Fagaceae, Poaceae, Polygonaceae, and Urticaceae. The result indicated that orbicules presented in all species. In species such as *Betula pendula*, orbicules have the ability as allergens as well as pollen (El-Ghazaly et al., 1995). Orbicules in *B. pendula* appear as spherical or irregularly shaped bodies and show considerable variation in size (2-4 µm). Almost the same as *B. pendula*, orbicules in Afican tulip tree were irregular and show considerable variation in size (<5 µm). From the results of morphological analysis using TEM and SEM, the morphological form of orbicules African tulip tree has the same shape as the allergen plant. Allergen plants have a small size of orbicules that has the potential to enter the human alveoli. This showed that African tulip tree have the potential to cause allergy.

An ultrastructure observation resulted that African tulip tree pollen could be potential as source for pollen allergen were supported by protein analysis. From 10 protein bands obtained between 10-70 kDa, it could be potential caused allergies, 4 bands with molecular weights of 55 kDa, 48 kDa, 39 kDa, and 29 kDa appear with very clear gene expressions and dark compared to the others. Among the four protein bands, the 55 kDa protein band has the darkest and clearest gene expression. The results showed that three molecules from African tulip tree were identified as African tulip tree allergens which have not yet been described. Referring to the names of allergens in IUIS (2014) and other literature, the possibility of a 55 kDa protein band is *polygalacturonase*, 48 kDa is a *pectase lyase*, 29 kDa is *beta-expansin* (Behrendt & Becker, 2001; de Weerd et al., 2002; de Dios Alchè et al., 2012). *Polygalacturonase, pectate lyase*, and *beta-expansin* are enzymes that needed for pollen germination

Polygalacturonase affects the degradation of pectin which was the main component of the cell wall (Swoboda et al., 2004). This protein was an allergen (Phlp 13) in *Phleum pratense* (IUIS, 2014). In corn pollen there were also proteins identified as *polygalacturonase* with a molecular weight of 50-60 kDa. These allergens could be related to the serum of patients with allergies and their reactivity reaches 50% (Taala et al., 2014). *Pectate lyase* was an allergen in the *Cryptomeria japonica* (Cry j 1, 48 kDa) and *Ambrosia artemisiifolia* (Amb a 1, 38 kDa) (de Dios Alchè et al., 2012). Function of *Pectate lyase* to facilitated pollen penetration (Marín-Rodríguez et al., 2002). *Beta expansin* was an allergen in *Paspalum notatum* (Pas n 1, 29 kDa), *Lolium perenne* (Lol p 1, 27 kDa) and *Anthoxanthum odoratum* (Ant o 1, 27 kDa) (de Weerd et al., 2002). *Beta expansin* has a molecular weight of 25-30 kDa with 250-300 amino acids (AbuQamar, 2014).

The function of *expansin* in pollen related to the invasion of pollen to the stigma and style (McQueen-Mason & Rochage, 1998). The most relevant African tulip tree allergen molecules belong to *polygalacturonase, pectase lyase*, and *beta-expansin*. From allergenic protein observation showed that African tulip tree potential for pollen allergen.

Acknowledgment

The authors would like to acknowledge Heri Prabowo, M.Sc., for giving a comments and encouragement.

References

AbuQamar, S. (2014). Expansins: Cell Wall Remodeling Proteins with a Potential Function in Plant Defense. *Journal of Plant Biochemistry & Physiology,* 2:1.

Behrendt, H., & Becker, W. M. (2001). Localization, Release and Bioavailability of Pollen Allergens: The Influence of Environmental Factor. *Current Opinion In Immunology,* 13, 709-715.

Bozzola, J. J., & Russell, L. D. (1998). *Electron Microscopy: Principles and Techniques for Biologist*. Second edition. USA, Jones and Bartlett Publishers.

Bradford, M. M. (1976). A Rapid and Sensitive Method for The Quantitation of Microgram Quantities of Protein Utilizing The Pcinciple of Protein-dye Binding. *Analytical Biochemistry,* 72, 248-254.

Chin, W. Y. (2003). *Tropical Trees and Shrubs*. Singapore, Sun Tree Publishing Limite.

D'Amato, G., Cecchi, L., Bonini, S., Nunes, C., Annesi-Maesano, L., Behrendt, H., Liccardi, G., Popov, T., & van Cauwenberge, P. (2007). Allergenic Pollen and Pollen Allergy in Europe. *Allergy.* doi: 10.IIII/j.1398-9995.2007.01393.x.

de Dios Alchè, J., Zafra, A., Jimènez-Lopèz, J. C., Morales, S., Castro, A. J., Florido, F., & Rodriguez-Garcia, I. (2012). Pollen Allergenicity is Highly Dependent on The Plant Genetic Background: The "Variety"/"Cultivar" Issues. In Jimènez-Lopèz, J.C (edits) *Current Insights In Pollen Allergens, Intech*.

de Weerd, N. A., Bhalla, P. L., & Singh, M. B. (2002). Aeroallergens and Pollinosis: Molecular and Immunological Characteristics of Cloned Pollen Allergens. *Aerobiologia*, 18, 87-106.

Dinis, A. M., Baptista, F., & Coutinho, A. P. (2007). Is The Quantity of Orbicules Released by Dactylis glomerata and Cynosurus echinatus (Poaceae) Big Enough to Play An Allergenic Role?. *Grana,* 46(3), 140-147.

Ebi, K. L., Burton, I., & McGregor, G. R. (2009). Biometeorology for Adaptation to Climate Variability and Change. *Springer Science + Business Media B.V*.

El-Ghazaly, G., Takahashi, Y., Nilsson, S., Grafstrom, E., & Berggren, B. (1995). Orbicules in Betula pendula and their Possible Role in Allergy. *Grana,* 34(5), 300-304. doi: 10.1080/00173139509429063.

Fatchiyah, Arumingtyas, E. L., Widyarti, S., & Rahayu, S. (2011). *Biologi molekuler: Prinsip Dasar Analisis*. Jakarta, Erlangga.

Gunawan, H., Takai, T., Ikeda, S., Okumura, K., & Ogawa, H. (2008). Protease Activity of Allergenic Pollen of Cedar, Cypress, Juniper, Birch and Ragweed. *Allergology International,* 57, 83-91.

International Union of Immunological Societies (IUIS) - Allergen Nomenclature Sub-Committee. (2014). *Allergen Nomenclature*. www.allergen.org

Kirmanto, D. (2012). *Pedoman Penanaman Pohon pada Sistem Jaringan Jalan*. Peraturan Menteri Pekerjaan Umum Nomor 05/PRT/M/2012, Kementrian Pekerjaan Umum. 38.

Laemmi, U. K. (1970). Cleavage of Structural Proteins During The Assembly of The Head of Bacteriophage T4. *Nature,* 227, 680-685.

Marín-Rodríguez, M. C., Orchard, J., & Seymour, G. B. (2002). Pectate lyases, Cell Wall Degradation and Fruit Softening. *Fruit Development and Ripening Special Issue,* 53(377), 2115-2119.

McQueen-Mason, S. J., & Rochange, F. (1998). Expansins in Plant Growth and Development: an Update on an Emerging Topic. *Plant Biology,* 1, 19-25.

Mrindula, P. A., Mahesh, P. A., Abraham, J. N., Agashe, S. N., Sitesh, R., & Vedanthan, P. K. (2011). Dolichandrone platycalyc: New Entomophilous Pollen – A Report on Pollen Sensitization in Allergic Individuals. *American Journal of Rhinology & Allergy,* 25(1), e34-8.

Prakashkumar, R., Mathew, P. M., & Ravindran, P. (1998). Studies on The Allergenicity of Nine Tropical Pollen Allergen. *Grana*, 37, 185-188.

Rengganis, I., Hartana, A., Guhardja, E., Djauzi, S., & Budiarti, S. (2008). Sensitivitas Terhadap Serbuk Sari Pasien Alergi Pernafasan. *Majalah Kedokteran Indonesia,* 58(9), 20-27.

Taala, L., Majd, A., Nourizadeh, M., & Pourpak, Z. (2014). Identification of The Allergenicity of Maize Pollen in Iran. *Archives of Iranian Medicine,* 17(7), 490-494.

Vanderplanck, M., Leroy, B., Wathelet, B., Wattiez, R., & Michez, D. (2014). Standardized Protocol to Evaluate Pollen Polypeptides as Bee Food Source. *Apidologie,* 45, 192-204.

Vigh-Conrad, K. A., Conrad, D. F., & Preuss, D. (2010). A Protein Allergen Microarray Detects Specific IgE to Pollen Surface, Cytoplasmic, and Commercial Allergen Extracts. *PLoS ONE,* 5(4): e10174. doi: 10.1371/journal.pone.0010174.

Vinckier, S., Cadot, P., & Smets, E. (2005). The Manifold Characters of Orbicules: Structural Diversity, Systematic Significance, and Vectors for Allergens. *Grana,* 44, 300–307.

Vinckier, S., & Smets, E. (2001). The Potential Role of Orbicules as a Vector of Allergens. *Allergy,* 56, 1129-1136.

Zeb, S., Perveen, A., & Khan, M. (2017). Analysis of Protein Profile and Pollen Morphology of Guaiacum officinale Linn. *Pakistan Journal of Botany,* 49(4), 1497-1500.