Full Length Research Paper

Pollen morphology of seven wild species of *Acacia* in Saudi Arabia

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Accepted 3 October, 2013

Pollen micromorphological characters such as pollen size, shape, number of associated monads, colpi and ornamentation of the tectum surface, of seven species of *Acacia* belonging to the family Mimosaceae were studied, using light microscope (LM) and scanning electron microscope (SEM). The study showed that the pollen size range from 42.1 to 69.4 µm, pollen shape is round to semi- round, the number of associated monads is 16 or 32, and the colpus is Y - H shaped. The tectum surface ornamentation among the species is variable, it is foveolate in *Acacia ehrenbergiana*; psilate-foveolate in *Acacia nilotica, Acacia laeta* and *Acacia negrii*; and micro-reticulate in *Acacia farnesiana, Acacia oerfota* and *Acacia tortilis* ssp. *raddiana*. An artificial key based on micromorphological characters is provided.

Key words: Mimosaceae, Acacia, pollen morphological characters, Saudi Arabia.

INTRODUCTION

Acacia Mill. belongs to the family Mimosaceae. It is widely distributed in tropical and subtropical regions (Elias, 1981). The subfamily Mimosoideae includes three tribes Acacieae Benth., Ingeae Benth. and Mimoseae Born. (Bentham,1842). Tribe Acacieae includes only a single genus Acacia Mill. as stated by Bentham (1875). The number of species recorded in this genus is about 1400 species widely distributed in tropical and subtropical regions. Hopper and Maslin (1978) recorded 600 to 900 species in Western Australia while Chaudhary and Al-Jawaid (1999) suggested 1100 species in the world. However, only 12 to 16 species have been reported in the Kingdom of Saudi Arabia (Chaudhary, 2000; Collenette, 1999; Migahid, 1996).

Acacia Mill. is an economically important genus, all parts of various Acacia species are used for one purpose or another as sources of food, fodder, fire-wood and a variety of natural products, such as wood, gum exudates, tannins and honey (Chaudhary, 1983; Springuel and Mekki, 1993; Al-Zoghet and Tag El-Din, 1995). Most of the *Acacia* species are of medicinal benefits to man and his livestock, e.g. *Acacia nilotica* produces arabic gum which is used for treating kidney diseases, and its pods are used for treating wounds and diarrhea (Elkhalifa, 1996).

Acacia pollen grains morphology has been studied by several investigators. Boulos L, 1983; Elias (1981) mentioned that the pollen characteristic features in Mimosoideae genera shows that they shed an individual persistent unit, as tetrad, octad, or polyad units, mostly of 16 and 32 monads. Guinet (1981) as recorded that the most structural pattern of the pollen grain is granular with common porate aperture, although, pollen grain with colporate and extraporate apertures are present, but the colpate aperture does not exist in pollen grains of the Mimosoideae. Jumah (1991, 1996) has reported spherical polyads of 16 grains in Acacia karroo; A. nilotica var. adansoni, A. nilotica var. tomentosa and Acacia polyacantha sub sp.

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| Specie | Pollen size LM (µm) | | (P/E) | Pollen shape | Number of monad | Type of colpi | Tectum surface | Sites and year |
|-----------------------------|---------------------|----------|-------|------------------|--------------------|---------------|-------------------|---------------------------------|
| | P | <u> </u> | | | or monau | | | CONSCION |
| A. ehrenbergiana | 49.8 | 49.2 | 1.01 | Rounded | 16 | Colporate | Foveolate | Al-Hada, 2005 |
| A. farnesiana | 67.9 | 69.4 | 0.98 | Semi- rounded | 32 | Colporate | Micro-reticulate | Al-Baha, 2001 |
| A. laeta | 42.1 | 44.1 | 0.96 | Rounded | 16 | Porate | Psilate-foveolate | Al-Baha -Bin Saad rood, 2005 |
| A. negrii | 45.4 | 44.8 | 1.01 | Semi- rounded | 32 | Colporate | Psilate-foveolate | Abha, 2003 |
| A. nilotica | 50 | 48 | 1.04 | Rounded | 32 | Colporate | Psilate-foveolate | Abha, 2006 |
| A. oerfota | 64.8 | 62.4 | 1.04 | Semi- rounded | 32 | Porate | Micro-reticulate | Abha, 2001 |
| A tortilis ssp. raddiana | 57 | 56.4 | 1.01 | Rounded | 32 | Colporate | Micro-reticulate | Beasha, 2003 |

Table 1. Sites, year of collection and pollen morphological characters of *Acacia* species under study. $P = Polar axis (\mu m)$, $E = equatorial diameter (\mu m)$, (P/E) = ratio of polar axis and equatorial diameter.

campylacantha. Guinet (1990) noted that the pollen structural symmetry was shared by some Mimoseae and Acacia. Kordofani and Ingrouille (1992) studied 14 species of Acacia reporting that the pollen grains in each polyad are16 except in one species which has 32 grains in each polyad. Fitzgerald et al. (1993) studied the development and initiation of cohesion between compound pollen grains of Acacia paradoxa and found special cases of pollen development. Robbertse (1974) examined the surface structures of pollen grains of Acacia giraffae and Acacia faidherbia in a study to clarify their taxonomic relationship. Perveen and Qaiser (1998) also studied the pollen grains of five species of Acacia in Pakistan and revealed that the pollen morphology differences of the subfamily Mimosoideae is significant at the generic and tribal level. Caccavari and Dome (2000) investigated morphological and structural characterization of pollen grains in 77 American species of Acacia and suggested that the pollen features can be used as a distinguishing factor of the generic restrictions for Acacia. Tantawy et al. (2005) also studied the pollen morphological characters in 14 species of Acacia in Egypt and reported that the morphological features of the pollen grains are indicative of generic and specific level of studied Mimosoideae. El Azab (2005) suggested that some Acacia species may be differentiated into different groups according to their pollen characters. Recently, Rajurkar et al., (2013) observed that A. nilotica and Acacia leucophloea polyads had 16 pollen grains and the pollen variations in their morphological characters for example, the shape, size, surface pattern and surface structure which was found to be significantly helpful at generic or specific level. The pollen of Acacia species in Saudi Arabia has limited investigations regarding the honey and allergy. So the aim of this study was to investigate in details the micro morphological characters of pollen grains of some Acacia species to show how far the pollen morphological

variations could be used to distinct between the studied species of *Acacia* which are growing naturally in Saudi Arabia.

MATERIALS AND METHODS

The pollen grains of 6 species and one subsp. of Acacia growing wildly in Saudi Arabia were obtained from specimens in the Herbarium of the Ministry of Agriculture and Water, Riyadh, Saudi Arabia (Table 1 and Figure 22). Pollen grains for light microscope (LM) examination were prepared using the usual acetolysis method (Erdtman, 1960; Moore et al., 1991) and mounted in either glycerin gelatine or glycerin. Observations were made using Olympus CH20 Microscope, and photographs were taken using Olympus BX4ITF with camera video TK-C1381EG. For scanning electron microscope (SEM) studies, pollen grains samples were run through an alcohol series: 50, 70, 80, 95, 100 % then mounted on the stubs using micro-pipettes. The stubs were coated usually to 30 nm with gold and then the pollen grains were ready for scanning (Punt, 1962). The representative pollen grains were photographed at various magnifications in a JSM-5800 LV (JOEL) scanning electron microscope. The measurements were based on 20 reading from each specimen. Descriptive terms were according to Moore and Webb (1978) and measurements for the pollen grains size of studied species were taken according to Erdtman (1960) [very small < 10 µm in polyad diameter; small 10-25 µm; medium (25-50) µm; large (50-100) µm; very large (100-200)].

RESULTS AND DISCUSSION

Representative pollen grains of 7 Acacia species are illustrated in Figures 1 to 21. Pollen morphological characters and measurements are provided in Table 1. The pollen shape is round in Acacia ehrenbergiana, Acacia laeta A. nilotica and Acacia tortilis ssp. raddiana (Figures 1, 3, 5, 7; Table 1), this result confirms that mentioned by Kordofani and Ingrouille (1992), Tantawy et al. (2005) and Rajurkar et al. (2013). Shape is semiround in Acacia farnesiana, A. negrii and A. oerfota



Figure 1-7. LM micrographs showing pollen shapes. 1: *Acaccia ehrenbergiana* pollen round; 2: *A. farnesiana*, pollen semi-round; 3: *A. laeta*, pollen round; 4: *A. negrii*; pollen semi-round; 5: *A. nilotica*, pollen round; 6: *A. oerfota*, pollen semi-round; 7: *A. tortilis* ssp. *raddiana*, pollen round. 400x.

(Figures 2, 4, 6; Table 1), and this in an agreement with report of Tantawy et al. (2005). The pollen size is large in A. farnesiana, A. oerfota and A. tortilis ssp. raddiana; and medium in A. ehrenbergiana, A. laeta, A. negrii and A. nilotica (Table 1). This result is almost similar to that reported by Tantawy et al. (2005) and Kordofani and Ingrouille (1992) on the pollen grains of A. tortilis ssp. raddiana and almost similar to that reported by Rajurkar et al. (2013) on the pollen grains of A. nilotica. The numberofassociatedmonadsare16-monadsinA.ehrenbergiana and A. laeta (Figures 8 and 12, respectively), and 32monads in A. farnesiana, A. negrii, A. nilotica, A. oerfota, and A. tortilis ssp. raddiana (Figures 10, 11, 14, 16, 18, 20 respectively and Table 1). This result is similar to that mentioned by Caccavari and Dome (2000) in their key of the pollen types and subtypes of American Acacia, and Rajurkar et al. (2013) on the pollen grains of A. nilotica. But it conflicts with that reported by Tantawy et al. (2005) since in the latter studies, all species have pollen grains with 32 monads (Figures 2, 4-7 and 10, 11, 14-21) except A. ehrenbergiana and A. laeta which have polyads (pollen grains) with 16 monads (Figures 1, 3, 8, 9, 12 and 13). Our result also disagree with that reported by Kordofani and Ingrouille (1992) about the presence of 16 monads in each polyad of A. nilotica and A. tortilis ssp. raddiana

(Figures 5, 16, 17 and 7, 20, 21, respectively) but agree with their finding on each polyad of A. ehrenbergiana with 16 monads (Figures 1, 8 and 9). The conflict in the number of monads in each pollen of A. nilotica and A. tortilis ssp. raddiana which grow in South West of Saudi Arabia and those grown in Sudan and Egypt may be due to the differences in environmental conditions in each country. The species of Acacia are characterized by pollen grains with colpi of Y and H-shape, that is, grooves- like at central and peripheral of the pollen grains provided with pores, this distinguishing character is used to identify the American Acacia species (Caccavari and Dome, (2000). However, the type of colpi is colporate in A. ehrenbergiana, A. farnesiana, A. negrii, A. nilotica, A. tortilis ssp. raddiana (Figures 9, 11, 15, 16, 19, 21 respectively and Table 1) and porate in A. laeta and A. oerfota (Figures 13, 19 and Table 1) this observation is in an agreement with that reported by Guinet (1981b). The tectum surface is foveolate in A. ehrenbergiana (Figure 9 and Table 1); psilate in A. laeta and A. negri (Figures 13, 15 and Table 1); psilate-foveolate in A. nilotica (Figure 16 and Table 1); and micro-reticulate in A. farnesiana, A. oerfota and A. tortilis ssp. raddiana (Figures 11, 19, 21 and Table 1). This result is similar to the observations of pollen of 16 or 32 monads confirmed that of Elias (1981)



Figure 8-21. SEM micrographs showing pollen structure. 8, 9: *Acacia ehrenbergiana*; pollen in polar view. 10, 11: *A. farnesiana*, pollen in polar view. 12, 13: *A. laeta* pollen in polar view. 14, 15: *A. negri* pollen in polar view. 16, 17: *A. nilotica* pollen in polar view. 18, 19: *A. orefota* pollen in equatorial view. 20, 21: *A. Tortilis* ssp. *raddiana*, pollen in polar view.

who indicated in his study that the pollen in Mimosoideae genera, shed single units of persistent tetrad, octad, or polyad units, mainly of 16 or 32 grains. We can divide the seven investigated species into two groups based on type of colpi and the number of polyads cells. The first group is polyad of 16 monads including A. ehrenbergiana and A. laeta which can be distinguished by the type of colpi, which is colporate in the pollen grains of A. ehrenbergiana (Figure 9 and Table 1) and porate in the pollen grains of A. laeta (Figure 13 and Table 1). The second group, polyad of 32 monads includes the other 5 species viz. A. oerfota, A. negrii, A. farnesiana, A. nilotica and A. tortilis ssp. raddiana. However, A. oerfota can be separated by its pollen grains with porate type (Figure 19 and Table 1) and the four remaining species are with colporate pollen grains (Figures 11, 13, 15, 21 and Table 1). Tectum surface of the pollen grains can also be used to divide the last four species into two groups, pollen grains with micro-reticulate surface includes *A. farnesiana* and *A. tortilis* ssp. *raddiana* (Figures 13, 21, respectively and Table 1) and pollen grains with psilate-foveolate surface includes *A. negri* and *A. nilotica* (Figures 15, 16, respectively and Table 1). Pollen grains size shows a great variation between the last four species, where *A. farnesiana* pollen size is 67.9-69.4 µm and *A. tortilis* ssp. *raddiana* pollen size is 57-56.4 µm, while *A. negrii* pollen size is 48-50 µm, which can be used to distinguish between them (Table 1).

Key based on the morphological characters of pollen grains of investigated *Acacia* species which grow naturally in Saudi Arabia as proposed:

I- Polyad, 16 monads



Figure 22. Collection sites of Acacia species in Saudi Arabia.

1-Type of colpi, Colporate *A. ehrenbergiana* 2- Type of colpi, Porate *A. laeta*

II- Polyad, 32 monads

- 1- Type of colpi, Porate *A. oerfota* 2-Type of colpi, Colporate
- A- Tectum surface, micro-reticulate

1- Pollen size (57-56.4 μm) *A. tortilis* ssp. *raddiana* 2 - Pollen size (67.9-69.4 μm) *A. farnesiana*

- B- Tectum surface, psilate-foveolate
- 1- Pollen size (48-50 μm) *A. nilotica*
- 2- Pollen size (44.8- 45.4 µm) A. negrii

ACKNOWLEGEMENTS

We would like to thank Dr. Nahed Wally, King Abdul Aziz University for valuable comments on the paper. Thanks are also due to Abdul Aziz Al-Jowaid, the Ministry of Agriculture and Water, Riyadh, Saudi Arabia for donation of specimens. This research was supported by a grant from the Research Center of the Center for Female Scientific and Medical Colleges, Deanship of Scientific Research, King Saud University.

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