Australian Journal of Basic and Applied Sciences, 3(1): 136-141, 2009
ISSN 1991-8178

Pollen Grain Micro Sculpturing and its Systematic Applications in Some Taxa of *Vigna Savi* (Leguminosae – Papilionoideae)

Wael, Taha Kasem

Botany and Microbiology Department, Faculty of Science, Al Azhar University, Egypt.

Abstract: Palynological studies on 11 taxa of *vigna savi* namely *V. radiata*, *V. trilobata*, *V. vexillata*, *V. caracalla*, *V. pubescens*, *V. unguiculata*, *V. membranacea*, *V. gracilis*, *V. luteola* and *V. oblongifolia*, was carried out using LM and SEM. Observations from the SEM investigation showed that the pollen grain varied in shape, size and surface ornamentation. Characters of pollen grain analyzed by NTSYS-PC program package using the UPGMA clustering methods grouped three distinct taxonomic levels. The first includes *V. racemosa*, *V.oblogfolia*, *V.gracillis* and *V. luteola*, the second included *V.vexllata, V.tribolata* and *V.radiata* and the third group included the 4 remainders (*V.caracalla, V membranacea*, V. *unguiculata* and *V. pubscenns)*. The relationships of the taxa as revealed by the produced dendrogram are discussed in relation to previous classification.

Key words: Leguminosae, papilionoideae, Pollen grain micro sculpturing, *Vigna savi.*

INTRODUCTION

The genus *Vigna savi* belongs to the family Leguminosae-Papilionoideae and Tribe Phaseolease which is made up of about 80-100 species that are tropical especially in Africa and Asia. (Mbagwu and Edeoga, 2006). In angiosperms, most important morphological features of pollen grains at the higher taxonomic levels involve their shapes, size, type, number, position. Structure of apertures, pollen wall (exine) sculpture and stratification. Several species of *Vigna* are of considerable economic importance in many developing countries. *Vigna* species are also valued as forage, cover and green manure crops in man parts of the world (Singh *et al*., 1970). Earlier studies of pollen morphology of *Vigna* are meager and they were conducted using only light microscopy (Erdtman, 1952; Mbagwu and Edeoga, 2006). The economic importance *Vigna savi* species exhibit a grow successfully in extreme environments such as high temperature, low rain fall and pore soils with few economic inputs (White, 1972). Most *Vigna savi* species such as *V. unguiculata*, *V. radiata* and *V. racemosa* called the pore-man's meat owing to their uses as a primary protein sources (Chopra and Swamy, 1975).Palynological attributes of plants have attracted the attention of many researchers in recent time. Palenology provides useful data for the intrageneric classification of the large genera (Nyananyo, 1985). Nyananyo and Olowokudejo (1986) also used seed coat and morphology and other palynological features of *Talinum* and *Calandrina* to produce a more acceptable classification of the species in these taxa. The variations in shape, aperture, polar unit symmetry and differences in wall sculpture of pollen grains have been used by many authors in the delimitation of various taxa (Aagwu and Uwakwe, 1992 and El-Ghamery, 2003). Aim of the work was to provide detailed pollen morphological and structural descriptions for taxa using LM, SEM and using obtained characters for the systematic of the taxa.

MATERIALS AND METHODS

11 taxa of *Vigna savi* were obtained from the International Livstock Center for Africa (ILCA) and from different localities in Egypt. List of the studied taxa is given in Table (1)

Pollen grains were collected after cultivation in Egypt to obtain pollen grain of buds and stored in refrigerator 3-5°C until used. Samples of taxa were acetolyzed according to Erdman's technique (Erdman, 1952). The acetolyzed and non acetolyzed samples were used for both light (LM) and scanning electron microscopy (SEM). For LM, semi-permanent slides were prepared using glycerin jelly, the prepared slid were examined and measurements were taken by using Zeiss light microscop with a pre-calibrated eye-piece micrometer. Polar axis (P) and equatorial diameters (E) of the pollen grains were measured. Colpus length and

Corresponding Author: Wael, T.K., Botany and Microbiology Dept., Faculty of Science, Al Azhar university, Egypt.

Table 1: Studied Taxa and localities.

No.	Species	Acc. No.	Orig.	Subgenus	Section1
1	*V. radiata* (L.) R. Wilczek	-	Egypt		Plectotropis
2	*V. trilobata,* L.	524	Ethiopia		Plectotropis
3	*V. vexillata,* A. Richard	54	Ethiopia	Plectotropis	Plectotropis
4	*V. caracalla* (L.) Verdc.	11057	Ethiopia	Sigmoidotropis	Caracallae
5	*V. pubescens,* Wilczek	41	Ethiopia	Vigna	Catiang
6	*V. unguiculata* (L.) Walp.	-	Egypt		Catiang
7	*V. membranacea,* A. Richard	7534	Ethiopia		Macrodontae
8	*V. gracilis* (Guill. & Perr.)	29	Ethiopia		Vigna
9	*V. luteola,* Benth.	6987	Ethiopia		Vigna
10	*V. oblongifolia,* A. Richard	30	Ethiopia		Vigna
11	*V. racemosa,* Hutch	45	Ethiopia		Vigna

the distance between two adjacent colpi (mesocolpi) were also measured and the ratio P/E was calculated to determine the shape class of pollen grains. For SEM, dried pollen grains were mounted onto clean stubs using double-sided adhesive, the samples were coated with a 30nm layer of gold using fine coat ion sputter JEOL-JFC-1100E ion -sputtering device. Then, the coated seeds were examined in SEM is operated at JEOL-JFC-5500V scanning electron microscopy, which operated at accelerated voltage of 15 kv at the scanning electron microscope unit, the Regional Center for Mycology and Biotechnology, Al-Azhar University. This study is dependent upon the using of a total pollen grain characters as a binary one (0 &1), on each of the 11 taxa. of the *Vigna savi*. Used characters and their states have been subjected to numerical analysis under a program using similarity and dissimilarity assessment percentage method (Rohlf, 1989). The method applied is based on cluster analysis by using an UPGMA (unweighted pair-group method with arithmetic means) dendrogram illustrating the interspecific relationships of the studied species as percent similarity.

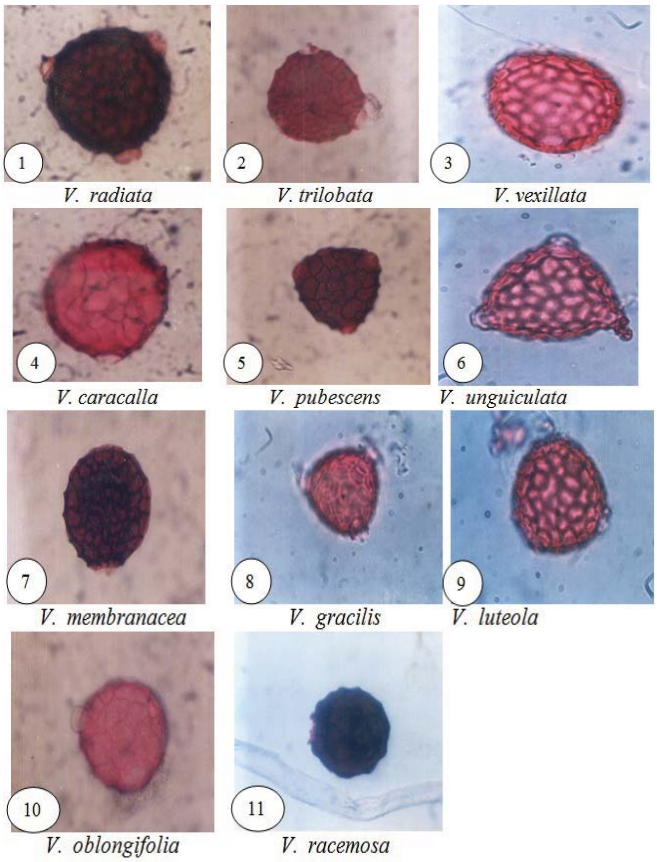


Plate I (Figs. 1-11): Light microscope (LM) of pollen grain of studied *Vigna savi* taxa (x=40).

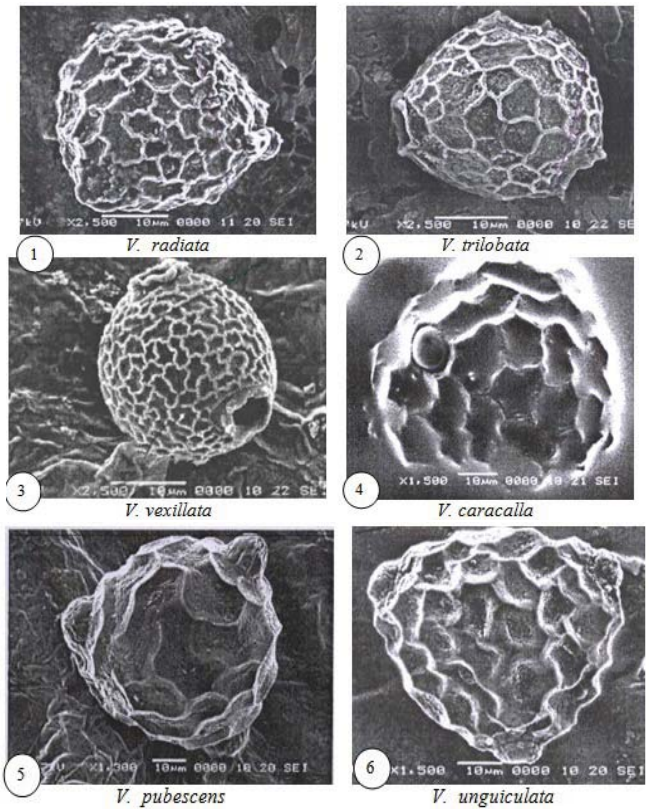
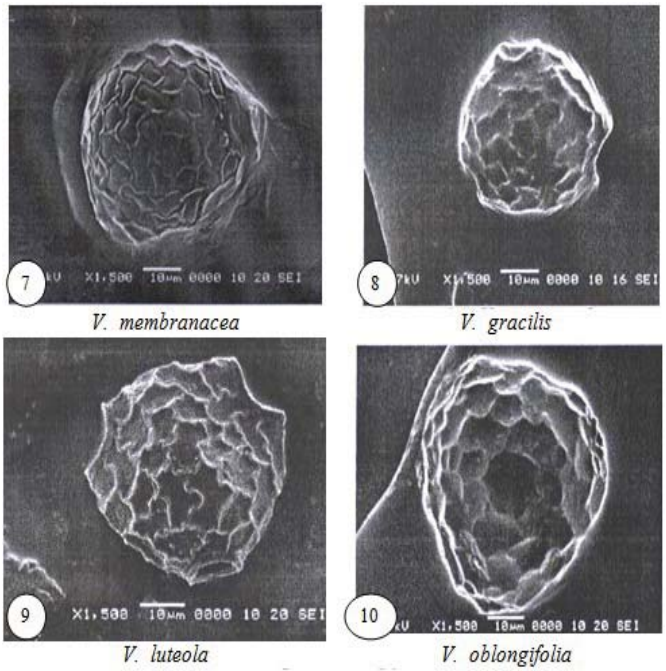
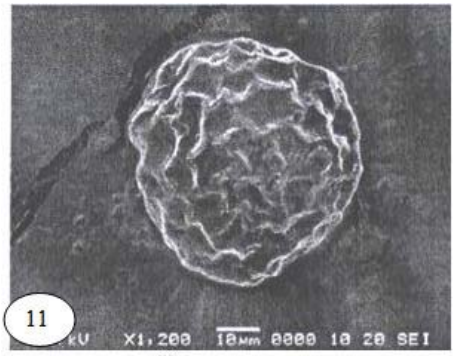


Plate II (Figs. 1-11): Scanning electron microscope (SEM) of pollen grain of studied *Vigna savi* taxa.





V. racemosa

(Cont.) Plate II (Figs. 1-11): SEM of pollen grain of studied *Vigna savi* taxa.

RESULTS AND DISCUSSION

Pollen grain Morphology of the *Vigna savi* showed diversity in shape, size, aperture types and exine characters. Results of LM fairly don't indicate the complete differentiation between the taxa (Plate I; Figs 1-11). The pollen grain apertures appear to be either dicolpate to dicolporate. Differences are significant and could be exploited for systematic purposes.

Table 2: Pollen grain characters for the studied taxa.

Characters Taxa	Polar axis (P)	Equatorial axis (E)	P/E	Aperture type	Sculpture type	General Shape
V. radiata	28.80 (27-31.5)**	27.60 (27-31.5)**	1.043	Prolate tricolpate	Reticulate Shape	Circular pollen grain
V. trilobata	30.23(30-32.5)**	33.52(30-35.5)**	0.90	Oblate tricolpate	Simplicolum-ellate Shape	Triangular pollen grain
V. vexillata	32.23(32-34.5)**	28.59(27-30.2)**	1.12	dicolpate	HomobrochateShape	Ovate pollen grain
V. caracalla	31.23(30-33.5)**	27.29(25-30.1)**	1.14	Prolate tricolpate	Duplicolum-ellate shape	Triangular pollen grain
V. pubescens	27.22(30-34.5)	25.26(22-28.6)**	1.07	Prolate tricolpate	Fossulate Shape	Triangular pollen grain
V. unguiculata	27.86(25-30.5)**	26.10(25-28.6)**	1.06	Prolate tricolporate	Echinolophate Shape	Circularpollen grain
V. membranacea	28.21(25-30.0)**	29.26(29-31.2) **	0.86	tricolpate	Reticulateshape	Obovate pollen grain
V. gracilis	32.20(29-35)**	34.25(32-36.9)**	0.84	Oblate tricolpate	Bireticulate Shape	Triangular pollen grain
V. luteola	38.28(30-40.2)**	40.21(38-41.0)**	0.95	Prolate tricolpate	VerrucateShape	Triangular pollen grain
V. oblongifolia	34.23(30-36.5)**	35.28(33-37.0) **	0.97	Oblate tricolpate	HeterobrochateShape	Triangular pollen grain
V. racemosa	26.58(22-35.0) **	23.28(22-24.0)**	1.14	Tricolpate	Ornate Shape	Circular pollen grain

**Significant at P ≥ 0.01

External morphology of pollen grain was studied with help of light microscope. Shape of pollen grain varied between the taxa studied. Sculpture varied between the studied species as follows:

V. radiata - pollen grain are dicolpate, circular shaped, surface has regulated pattern and surface shows a reticulum with brochi of the same sizes (Table2, Plate II, Fig. 1).

V. trilobata-oblate tricolpate, triangular, surface shows regulate ornamentation consisting of irregularly arranged, winding, or angular rounded muri of varying thickness, which do not form a distinct reticulum, but rather a maze-like pattern (Table2, Plate II, Fig. 2).

V. vexillata - Prolate tricolpate, ovate, a network-like pattern consisting of lumina or other spaces wider than 1µm bordered by elements narrower than the lumina present (Table2, Plate II, Fig. 3).

V. caracalla-Prolate tricolporate, triangular shape has regulated pattern and surface shows a lophate pollen grain with echinate ridges (Table2, Fig. 4).

V. pubescens - Prolate tricolpate, triangular form, surface has a feature of ornamentation consisting of an elongated regulate echinate groove on the surface of pollen (Table2, Plate II; Fig.5).

6- *V. unguiculata*-Prolate tricolpate, subspheroidal has regulate ornamentation and surface shows columellae in two rows under each murus (Table 2, Plate II, Fig. 6).

7- *V. membranacea*-tricolpate, obovate, their surface has regulate ornamentation and such ornamentation consisting of reticulate form with folded over pleats in word it (Table 2, Plate II, Fig. 7).

8- *V. gracilis*-oblate tricolpate,triangular shaped, regulate ornamentation and a two layered reticulum consisting of a suprareticulum supported by a microreticulate tectum has been recorded (Table 2, Plate II, Fig. 8).

9- *V. luteola*-Prolate tricolpate, has triangular pollen grains; their surface presents regulate ornamentation and a presence of wart-like sexine element, more than 1µm wide, that is broader than it is high and is not constricted at the base (Table 2, Plate II, Fig. 9).

10- *V. Oblongifolia-oblate* tricolpate, triangular shaped; surface reveals regulate ornamentation and reticulum with brochi of different sizes (Table 2, Plate II, Fig. 10).

11- *V. racemosa*-tricolpate, circular shaped pollen grain, surface has regulated ornamentation in addition to presence reticulate ornamentation consisting of broad, curved muri and lumina that are often anastomosing (Table 2, Plate II, Fig. 11).

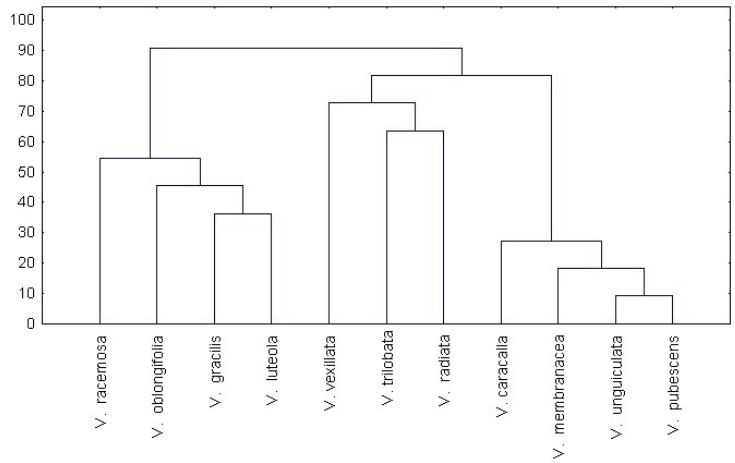


Fig. 12: UPGMA-dendrogram based on characters illustrating similarity & dissimilarity distances between the studied species

Taxa of *Vigna savi* have been an average dissimilarity percentage of 90 % (Fig.12). At this level the 11 selected taxa of the *vigna savi* were delimited into three distinct groups. The first included *V. racemosa*, *V. oblogfolia*, *V. gracillis* and *V. luteola* (55% dissimilarity percentage) such taxa related to section *Vigna*, the second included *V. vexllata, V. tribolata* and *V.radiata* (75% dissimilarity percentage) which related to Plectotropis and the third group included *V. caracalla, V. membranacea*, *V. unguiculata* and *V. pubscenns* (25% dissimilarity percentage). Such taxa are related to section Caracallae, Macrodontae and Catiang, respectively. From the dendrogram, the highest similarity was found between *V. unguiculata* and *V. pubscenns* (10 % dissimilarity percentage) which related to section Catiang. Such two species has achinate sculpture on their surface. On the other hand the ratio of P/E was 1.06 and 1.07, respectively (Table 3).The present work differed with earlier studies of Mbagwu and Edeoga (2006) which suggested that *V. unguiculata* was grouped with *V. racemosa*, based on their light microscope studies of 8 taxa of *Vigna savi* in Nigeria. Our results are agreed with their results of *V. vexillata* and *V. trilobata* are grouped in single link. On the other hand, *V. vexillata* split than other taxa of the same section, because of presence of dicolpate structure on their surface .In the first group *V.gracilis* and *V. luteola* has a high similarity percentage than other taxa of the section *Vigna savi*; such two species have fairly similar results in shape and size characters.

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