International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

A Comparative Pollen Morphological Study of Some Selected Members of the Family Verbenaceae and Lamiaceae

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Abstract
The present study aims to compare some selected members of Verbenaceae and Lamiaceae by giving special emphasis on their pollen morphology. The different taxa under investigation were *Vitex negundo* L., *Clerodendron infortunatum* L., *Duranta plumier* Jacq., *Lantana camara* L., *Leucas aspera* Spreng., *Plectranthus scutellarioides L.* and *Ocimum sanctum* L. Pollen morphological analysis was done using Scanning Electron Micrographs. Although these families show some similarities, they differ in many features such as phyllotaxy, inflorescence type, style, aperture number and type of pollen.

Key words: Verbenaceae, Lamiaceae, Pollen Morphology

Introduction

The family Verbenaceae is closely allied to Lamiaceae and to Boraginaceae and Scrophulariaceae to some extent. Verbenaceae and Lamiaceae has been placed under the order Lamiales by Bentham and Hooker (1862-1883)) and in the order Tubiflorae by Engler and Prantl (1924), Rolf M.T. Dahlgren (1980), and Arthur Cronquist (1988) placed the families in the order-Lamiales. .In the APG III classification Verbenaceae and Lamiaceae has been placed under the order Lamiales which is included under the Asterids clade of Eudicots. According to APG III many genera which were included in Verbenaceae by earlier classifications comes under the family Lamiaceae.

Verbenaceae is predominantly a tropical family, and comprises 75 genera with over 3000 species (Heywood 1975). Recent phylogenetic studies have shown that numerous genera traditionally classified in Verbenaceae belong in Lamiaceae. The new narrowly circumscribed Verbenaceae family includes some 35 genera and 1200 species (APG III). The members inhabit a wide range of habitats, mangroves to deserts and constituents of tropical forest to weed communities. The growth form varies from small trailing herb (*Phyla nodiflora*) to woody trees (*Tectona grandis*). Family contains trees, shrubs, and herbs notable for heads, spikes, or clusters of small flowers, many of which have an aromatic smell. The leaves are usually opposite or whorled and sometimes aromatic. The family includes economically important plants.

Lamiaceae is a large cosmopolitan family. The enlarged Lamiaceae contains about 236 genera and has been stated to contain 6,900 to 7,200 species (APG III), but the World Checklist lists 7,534. The original family name is Labiatae, so given because the flowers typically have

petals fused into an upper lip and a lower lip (*labia* in Latin). Plants contain square stalks with opposite leaves and usually aromatic. Flowers are usually bisexual and verticillastrate (a flower cluster that looks like a whorl of flowers but actually consists of two crowded clusters). Stamens 4, Didynamous, the two pairs usually inserted at different levels in the corolla tube. The fruit is commonly a dry nutlet. The Lamiaceae is an economically important family.

Pollen grain constitutes a vital plant unit, invariably present in all sexually reproducing angiospermic taxa, and it performs the physiological function of reproduction. The pollen of related families and genera are usually of more or less of same type. There are many morphological characters which are used for classification of pollen and impart great value in angiosperm taxonomy. The position, shape, structure and number of aperture are of taxonomic significance.The recent introduction at Scanning Electron Microscopy (SEM) has proved to be a useful tool for palynological studies with increased accuracy and precision. This has opened up possibilities for better understanding of the exine ornamentation pattern and enabled application of exine features in studies involved systematic relationships of microtaxa particularly sub species, varieties, cultivar, cytotypes, bio forms etc. (Ravikumar and Nair, 1979).

The exine of pollen is endowed with such stable morphological characters which are genetically fixed and do not get influenced by the environmental fluctuations. These characters are specific for different genera and may vary from species to species. Diversity of the aperture types and multiplicity of the exine sculpturing make them highly useful characters of taxonomic value (Nair, 1965, 1966).

From the studies conducted over years, it is now realised that the morphology of pollen as contained in the exine wall may be rsesolved into five groups of characters, namely: germinal aperture, exine ornamentation, exine strata, size and shape in the order of their importance. A combination of these features provides a particular taxon an entity in itself. This is the priniciple on which the application of pollen morphology in taxonomy and phylogeny is based. In studies involving hybrids and their parents, the pollen grains have provided interesting data on the inheritance patterns, relating to exine ornamentation and the germinal aperture (Ravikumar, 1979).

The present study aims to determine the extent of pollen diversity within the families and to discuss the systematic relationship of the group using additional data of pollen morphology

Material and methods

The plants belonging to both the families were collected in and around the campus of Sree Narayana College, Cherthala, Kerala, India (**Table.1**). Photographs were taken using digital camera. The collected plants : Clerodendrum infortunatum L.,Duranta plumieri Jacq.,Lantana camara L.,Vitex negundoL.,Leucas aspera Spreng.,Ocimum sanctum L.,Plectranthus scutellarioides L. were watered and stored in polythene bags to keep the materials fresh untill observation.

For pollen morphological studies, pollen grains were collected from mature flower buds. Pollen grains were examined using both light microscopy (LM) and Scanning Electron Microscopy (SEM). The terminology used to categorize pollen grains according to shape and size was that used by Walker and Doyle. For SEM, pollen samples were washed with distilled water, dehydrated in an ethanol series and air dried on Aluminium stubs. Then, sputter coated with Gold–Palladium by a Quorum SC7620 sputter coater. Subsequently these were examined and photographed with a TESCAN VEGA 3 SBH scanning electron microscope operated at 8-10 V. Pollen shape, pollen diameter and exine sculpturing were

examined. This work was carried out in Physics Research Laboratory, Maharajas College, Ernakulam, Kerala. India.

Table 2. Pollen size classes according to Walker and Doyle(1975).

Sl. No.	**Size classes**	**Longest axis**	**Shape classes**	**P/E* x 100**
1	Minute grain	<10µm	Per oblate	< 50
2	Small grain	10-24µm	Oblate	50-75
3	Medium sized grain	25-49µm	Sub oblate	75-88
4	Large grain	50-99µm	Oblate spheroidal	88-100
5	Very large grain	100-199µm	Spheroidal	100
6	Gigantic grain	>200µm	Prolate spheroidal	100-114
7			Sub prolate	114-133
8			Prolate	133-200
9			Perprolate	> 200

Result and discussion

Table 3. Pollen morphology of the members of Verbenaceae & Lamiaceae

Name of taxon	**Aperture morphotype**	**Shape**	**Pollen diameter** Polar (µm)	**Pollen diameter** Equatorial (µm)	**Size**	**Exine ornamentation**
Vitex negundo L.	3 Zonocolpate	Prolate	22.80	16.58	Small	Micro reticulate
***Clerodendron infortunatum* L**	3 Zonocolpate	Oblate Spheroidal	47.15	49.47	Large	Spinate
***Duranta plumieri* Jacq.**	zonocolporate	Prolate	37.52	19.57	Medium	Pislate

Lantana camara L.	3 Zonocolpate	Prolate Spheroidal	27.93	26.65	Medium	Pislate
***Leucas aspera* Spreng.**	3 zonocolporate	Prolate	29.07	21.70	Medium	Reticulate
***Plectranthus scutellarioides* L.**	6 zonocolpate	Prolate	28.77	21.49	Medium	Micro reticulate
***Ocimum sanctum* L.**	6 Zonocolpate	Prolate	43.94	30.98	Medium	Lipho reticulate

In the present investigation a comparative pollen morphological study was done in seven different taxa belonging to family Verbenaceae and Lamiaceae. The different taxa under investigation are *Vitex negundo*, L., *Clerodendron infortunatum*, L., *Duranta plumieri*, Jacq., *Lantana camara*, L., *Leucas aspera*, Spreng., *Plectranthus scutellarioides*, *L.* and *Ocimum sanctum*, L.

Pollen grains form a unique entity both with regard to its form and function. Although tiny in size, it serves as the sole medium through which the entire male genetic attribute are transmitted to the next generation. The structural features of pollen grains are regarded as a more dependable tool in studies of comparative morphology that leads to conclusion in plant taxonomy, phylogeny and evolution than those of other vegetative characters (Saad, 1972; Nair, 1974). The unique architectural features of exine are genetically controlled and stable. The exine of pollen grain embodies morphological characters which are broadly categorized into aperture, exine ornamentation, pollen size and shape in the order of importance in its application in plant taxonomy and phylogeny (Nair, 1966).

Pollen unit is the grouping in which pollen is found at maturity within the anther (Walker and Doyle, 1975). Different types of pollen unit occurring in angiosperm families are monads, dyads, tetrads, polyads, massulae and pollinia. Monads are considered as the simplest in evolutionary line and polyads are the most advanced. Presently all of the taxa belonging to family Verbenaceae and Lamiaceae possess grains as monads.

Apertures are delimited, thin walled areas in the exine. Functionally they are meant for protection, ion exchange and germination (Thanikaimoni, 1977). Aperture morphoform provides one of the best taxonomic characters especially at the higher level of taxonomy. The aperture may be either in the form of a furrow (colpus- colpate grain) or circular (pore-porate grain). Phylogenetically the colpate condition is primitive in angiosperms and here the aperture is a wide open furrow in which the ectocolpium and endocolpium are congruent. The colpate, porate and pororate are evolved conditions formed by the reduction and modification of the size of the ecto and endocolpium. According to Nair (1988), as regard to position of the pollen aperture, proximal position is considered to be most primitive while others such as distal, zonal and global are the derived and advanced conditions. In the presently investigated species of the family Verbenaceae, all the pollen grains were found to possess colpate or

colporate aperture. The number of aperture is found to 3. With regard to position, all the species show zonal aperture. Among all the taxa studied common apertural form is 3-zonocolporate or 3-zonocolpate. In studied species of the family Lamiaceae, all the pollen grains were found to possess colpate aperture. The number of aperture is found to vary from 3 to 6 of which 6 aperturate grains are common. Among all the taxa studied common apertural form is 6-zonocolpate.

The sculpturing (ornamentation) on the outer surface of exine is of considerable phylogenetic importance. Generally exine sculpturing is of two broad categories, the exerscence type and depression type. The exerscence type (spinulose, spinose, baculate, clavate, verrucate, tuberculate, granulate) which is less specialized, while the latter is advanced (psilate, reticulate, foveolate, scrobiculate, fossulate, striate) (Nair, 1978). A critical observation of the scanning electron micrographs of the exine surface of the different Verbenaceae members revealed less diversity. All the pollen grains investigated have depression type of ornamentation. Of the different types of depression types of ornamentation the most predominantly occuring type is psilate. *Duranta plumieri* Jacq. and *Lantana camara*, L. also possess psilate type of ornamentation.(Table). According to Wodehouse (1935), pollen grains with thick and heavily ornamented exine have been considered to be primitive, while those with thin unornamented (psilate) or lightly ornamented exine are considered to be advanced.

The pollen size and shape are the tertiary characters which are of little value in applied taxonomy due to their least significance in palynological considerations. The size and shape may be affected by process of acetolysis, so this cannot be considered as a reliable character (Nair, 1970). But size and shape are considered in many families either in generic or species level for correct placement in the group. Pollen size classes were suggested by Walker and Doyle(Table). In the present study, all taxa of both families possess medium sized grains except *Vitex negundo*, L. (small size) and *Clerodendron infortunatum*, L. (Large size). The average size of the pollen in Verbenaceae varied from 17-23µm in *Vitex negundo*, L. to 50-48µm in *Clerodendron infortunatum* L. In *Lantana camara*, L. the size of the pollen was observed to be 28-27µm, whereas in *Duranta plumieri*, Jacq. it was 20-38µm. The average size of the pollen in Lamiaceae varied from 21-29µm in *Plectranthus scutellarioides, L.* to 31-44µm in *Ocimum sanctum*, L. In *Leucas aspera*, Spreng. The size of the pollen was observed to be 22-29µm(Table).

Based on the shape, the pollen grains can be grouped into peroblate, oblate, sub oblate, oblate-spheroidal, spheroidal, prolate, sub prolate, euprolate and perprolate(Table). During the present study the shape of the pollen grains was found to be prolate in *Vitex negundo*, L., oblate spheroidal in *Clerodendron infortunatum* L., prolate in *Duranta plumieri*, Jacq., and prolate spheroidal in *Lantana camara*, L. All the taxa under study in Lamiaceae posses prolate shaped pollen grains(Table).

Conclusions

Vitex negundo, L. possess aromatic quadrangular hairy stem; corolla tubular, bilipped, zygomorphic; stamens epipetalous, didynamous, dorsifixed anther, and stigma bifid. These characters were similar to that of Lamiaceae features. But, *Vitex negundo*, L showed some variation in habit and leaf. It was small tree with trifoliate leaves.

Pollen morphology of *Vitex negundo*, L. has showed similarity with present studied Lamiaceae members. They have monad pollen grains, aperture type was colpate, pollen shape prolate and exine ornamentation was reticulate.

Taxonomically *Clerodendron infortunatum*, L. showed both similarity and dissimilarity with members of Lamiaceae. Similar features were aromatic shrub, quadrangular hairy stem, leaves simple, stamens epipetalous and didynamous, and stigma bifid. Dissimilar in type of inflorescence. Verticillaster is common type in Lamiaceae but *Clerodendron infortunatum*, L. possessdichasial cyme.

Pollen morphology of *Clerodendron infortunatum*, L. also different from Lamiaceae. Common aperture number of L amiaceae was 6 but *Clerodendron infortunatum*, L. has 3. Shape of pollen also varied. *C. infortunatum*, L. possess oblate spheroidal shape but Lamiaceae possess prolate. Lamiaceae members were medium sized grains but it possess large sized grains. Exine ornamentation also different. *C. infortunatum*, L. have excrescence type spinate nature but Lamiaceae possess reticulative.

Duranta plumieri, Jacq., *Lantana camara*, L. showed clear variatios in pollen morphology from Lamiaceae members.

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