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Original Research Article

PALYNOLOGICAL STUDIES ON SELECTED TROPICAL ACANTHACEAE AND SCROPHULARIACEAE

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Abstract: Pollen grains are the microspores inangiosperms, which in acanthaceae and scrophulariaceae varied size from fine to coarse structures and their size can range from 10 to 100 micrometer. As sucrose is considered to serve energy and osmotic for pollen germination, in the present pollen study sucrose based poller germination studies was performed in 20 plant species involved in acanthaceae and scrophulariaceae family. There is remarkable diversity of pollen morphology in various characters such as pollen size, shape, length and width of the pollen tubes identified from the germination study. Morphological characters of pollen grains also can be useful in the studies of plant systematic and palynological characters are used as a tool for diagnosis of a new species.

Key words: - Palynological, Acanthaceae and scrophulariaceae.

Introduction: Acanthaceae and scrophulariaceae of the series bicarpellatae belong to the order personals. Order personales is characterized by features such as herbaceous or shrubby habit plants are mostly herbs, under shrubs, simple leave, irregular hypogynous flowers, stamens epipetalous, less than the number of corolla lobes. The fruits are usually simple capsules.

Acanthaceae is an interesting and a variable family. Being the third largest tropical family, it occupies seventh position in the order of dominance in India. This clearly indicates the complexity and diversity of the family, which comprises about 250 genera and 2,500 species (Willies, 1973). The members of the family are concentrated in the tropical parts of the world, while some extend to the temperate regions. The four main centres of distribution are Africa, Central America, Brazil and Indo-Malaysia. In India, it is represented by about 49 genera and over 500 species (Clarke in Hooker, 1884-1885)

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along the mountains of south west India and some in the tropical and sub-tropical Himalayas. Scrophulariaceae embrace some 3,000 species and 200 genera (Willies, 1966), mostly of the herbaceous plants distributed throughout the world. The family is represented by about 275 species in India. The members are characterized by with irregular corolla, reduction in the number of calyx and corolla, stamens two four, presence of parasitic forms – an additional advanced character of the family as compared to Solanaceae and zygomorphic flowers.

The role of cytology and palynology in the systematic of acanthaceae and scrophulariaceae is well exemplified through exhaustive literature both modern and ancient (Radlkofer, 1883; Lindau, 1895; Bhaduri, 1944; Grant, 1955, Chaubal, 1966; De, 1966 & 1968; Saad, 1972; Saggoo and Bir, 1983; Daniel, 1988, 1990; Furness, 1986; Immelman, 1989 and Hilsenbeck, 1990a).

'Pollen grains' or 'Microspores' are comparatively simple haploid organisms. The study of pollen grains is called, 'Palynology', which is derived from the Greek words – 'to strew' or to sprinkle fine meal' cognate with the latin 'pollen', - four or dust. Little do we realize that the beauty of the nature around us is due to these tiny, yellowish or white powdery masses that they seem to be the store house of the gametes which are responsible for the propagation of plants around us. As pointed by (Brewbaker1967) – the rapid rate of germination and the quick process of fertilization are mainly responsible for their success in xeric land.

Materials and Methods: The present work was based on selected taxa belonging to acanthaceae and scrophulariaceae. The names of taxa and their collection are given in Table 1. The following aspects were studied.

(i)Fresh pollen: Temporary mounts of fresh pollen were made in water and examined the shape, under microscope. Measurements of pollen were taken with the help of micrometer.

(ii) Pollen culture: The pollen germination was studied during the morning hours pollen grains were cultured using sucrose solutions of varying concentrations, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 5.5%, 6%, 65%, 7%, 7.5%, 8%, 3.5%, 9%, 9.5% & 10%. The solution was taken on cavity slides and fresh pollen grains were dusted into the solution using a clean brush. The solution was covered with a cover glass and kept undisturbed germination of the pollen was observed at intervals 30 minutes and was tabulated. The length of the pollen tube was measured.

(iii) Estimation of pollen sterility: The pollen grains collected were stained in 1% acetocarmine and glycerine (1:1 mixture). The prepared slides are examined under microscope and stained pollen grains were counted as fertile one and unstained and partially stained pollen grains are sterile. Percentage of fertility and sterility were calculated.

Table 1 – List of taxa under study

No.	Name of the Plants (Acanthaceae members)
Sp1	*Adhato dabeddomei*, Clarke
Sp2	*Andrographis paniculata*, Nees
Sp3	*Cross andraundulaefolia*, Salish
Sp4	*Ecboliu mlinnaenum*Kurz Var. *latevirens*
Sp5	*Ecbolium linnaenum*KurzVar, *rotundifolium*
Sp6	*Eranthemum nervosum*, R. Br.
Sp7	*Hydrophila augustifolia* (Schum). Heine
Sp8	*Justicia betonica*, Linn.
Sp9	*Justicia procumbens*, Linn.
Sp10	*Ruelia tuberosa*, Poir
	Name of the Plants(Scrophulariaceae)
Sp11	*Ilysantheshys sopioides*,Benth.
Sp12	*Ilysanthes minima*,Benth.
Sp13	*Ilysanthes serrata*, Urban
Sp14	*Limnophila heterophylla*, Benth.
Sp15	*Scopari adulcis*, Linn.
Sp16	*Toreni abicolor*,Dalz.
Sp17	*Toreni acourtallensis*, Gamble
Sp18	*Toreni aparviflora*, Ham.
Sp19	*Vandeli acrustaceae*,Benth
Sp20	*Vandeli apyxidaria*, Maxim

Results

***Adhatoda beddomei*, Clarke:** Plant is a very large shrub, with rather small flowers in short head, turns, black on drying. Branches are glabrous with large leaves (Malayalam – Chittadalodakam). The leaves and roots medicinally important.

Pollen morphology: The pollen grains are yellow in colour. They are oblong. The length of the pollen grains is 21.42µ and width 14.28µ.

Pollen germination: Pollen grains showed maximum germination at 6% sucrose solution. Length of the tube measured 31.4µ and width 8.56µ.

Pollen fertility: The percentage of fertility obtained in acetocarmine method was 91.67% and percentage of sterility was 8.33%.

***Andrographis paniculata*, Nees.:** Plant is an erect annual herb with pink corolla, darker on the lower lip, the capsule usually 12-seeded. A bitter plant used in medicine. Referred to as 'King of bitters', Kiriath (Malayalam).

Pollen morphology: Pollen grains are yellow in colour. They are spherical of diameter 14.28µ.

Pollen germination: Pollen grains showed maximum germination at 6% sucrose solution. Length of pollen tube was 21.42µ and diameter 7.14µ.

Pollen fertility: The percentage of fertility noticed in acetocarmine method was 93.44% and percentage of sterility was 6.56%.

***Crossandra undulaefolia*, Salish.:** Plant is a pretty under shrub up to 3 ft. high with orange – yellow flowers and whorled leaves, lanceolate, acute, decurrent at base, up to 6 inches long, 2 inches broad often called as 'Kanakambaram' or Manjakurinji (Malayalam). Capsules are highly aphrodisiac.

Pollen morphology: Pollen grains are oblong of length 22.84µ and of width 9.99µ.

Pollen germination: The highest percentage of germination was observed in 5% sucrose solution. The pollen tube attained a length of 28.56µ and diameter of 5.71µ.

Pollen fertility: The percentage of fertility noticed in acetocarmine method was 94.63% and the percentage of sterility was 5.37%.

***Ecbolium linneanum*, Kurz. Var. *Laetevirens*:** Plant is a low shrub with bluish – green corolla, the bracts obtuse or slightly acute, the spikes short and leaves small and pubescent. The plant bears the vernacular name 'Odiyamadanta' (Malayalam) commonly known as 'Kurinji'. The leaves are used in medicine.

Pollen morphology: Pollen grains are oblong of length 19.97µ and diameter of 7.14µ.

Pollen germination: The highest percentage of 13.3% germination was observed in 5% sucrose solution. The maximum length of the pollen tube was 34.27µ and a diameter of 14.28µ.

Pollen fertility: The percentage of fertility noticed was 93.21% and percentage of sterility was 6.79%.

***Ecbolium linneanum*, Kurz. Var. *Rotundifolium*:** A low shrub with bluish green corolla, leaves small, sessile, ovate, bracts shortly cristate.

Pollen morphology: Fresh pollens yellow in colour grains are oblong, 19.9µ in length and 5.99µ in width.

Pollen germination: Percentage of germination was maximum at 5% sucrose solution with 15.5% germination. The length of the tube measured 12.85µ long and 4.2µ wide.

Pollen fertility: The percentage of fertility was 95.35% and percentage of sterility 4.65%.

***Eranthemum nervosum*, R.Br.** A pretty shrub reaching 5 ft in height with bright blue flowers in short bracteate spikes in large terminal panicles.

Pollen morphology: The fresh pollen grains are yellow in colour. Grains are spherical of diameter 28.56µ.

Pollen germination: Highest percentage of germination was found in 5.5% sucrose solution and lowest in 3.5% sucrose solution. Length of the tube was 21.42µ and diameter of 17.136µ.

Pollen fertility: the percentage of fertility was 96% and percentage of sterility 4%.

.*hygrophila augustifolia*, schum.

Often called as 'Vayulchulli' (Malayalam). A spinous herb, growing abundantly in rice fields and marshy places. The whole plant widely used in Ayurvedic systems, considered to be cooling, diuretic and strengthening. Anti-microbial activity also reported.

Pollen morphology: The fresh pollen grains are yellow in colour. Grains are oblong, 19.99µ long and 14.28µ wide.

Pollen germination: Pollen grains showed maximum germination at 5.5% sucrose solution with 20% germination. Length of the tube was 15.708µ and diameter of 5.712µ

Pollen fertility

The percentage of fertility 93.25% and percentage of sterility 6.75%.

***Justicia betonica*, Linn.:** Often referred as 'VellaKurinji'. The plant grows to a shrub with terminal white inflorescence with green nerved bracts.

Pollen morphology: Pollen grains yellow in colour. Grains are oblong with 14.28µ long and diameter 9.99µ.

Pollen germination: The percentage of germination was maximum in 6% sucrose solution with a maximum of 58% germination. Tube grew up to a length of 17.136µ and a width of 11.424µ.

Pollen fertility: The percentage of fertility 83.68% and sterility 16.32%.

***Justicia prostrata*, Linn.:** A small pale prostrate plant, the branches long and diffusely spreading from a stout rootstock sometimes almost woody, the flowers pale pink, the capsules small and minutely puberulous, the seeds minutely tuberculate. The leaves are small and usually orbicular or ovate, sometimes slightly lanceolate.

Pollen morphology: Fresh pollen grains are oblong of 17.13µ long and 5.712µ wide.

Pollen germination: Maximum percentage of germination was at 6% sucrose solution of 14.27% germination.

Pollen fertility: The percentage of fertility 88.8% and percentage of sterility 11.2%.

***Ruellia tuberosa*, Poir.:** A diffuse under shrub with solitary pale blue or purple flowers. Capsule slightly pubescent.

Pollen morphology: Fresh pollen grains yellow in colour. Grains are oblong and lightly curved or bean shaped of 24.27µ long and 11.42µ wide.

Pollen germination: High percentage of 10% germination was observed at 6% sucrose solution. Maximum length of pollen tube attained was 12.852µ and width of 4.284µ.

Pollen fertility: The percentage of fertility 94.4% and percentage of sterility 5.6%.

***Ilysanthes hyssopioides*, Benth.:** A straggling slender herb reaching 18 inches in height, the flowers pale blue or white with darker markings, sometimes nearly 1 inche long, the staminodes large and clavate, obscurely bilobed, glandular.

Pollen morphology: Fresh pollen grains are spherical. Grains are yellow in colour of diameter 7.14µ.

Pollen germination: The pollen grains showed 20% germination in 5% sucrose solution. The tube grew and attained a length of 15.708µ and width of 2.856µ.

Pollen fertility: The percentage of fertility 88.23% and percentage of sterility 11.77%.

***Ilysanthes minima*, Benth.:** A minute erect herb with rather large yellow flowers.

Pollen morphology: Fresh pollen grains yellow in colour. Grains are spherical of 5.712µ diameter.

Pollen germination: Highest percentage of germination of grains 22% in 5% sucrose solution. The tube attained a length of 14.28µ and width of 2.856µ.

Pollen fertility: The percentage of fertility 86.35% and percentage of sterility 13.65%.

***Ilysanthes serrata*, Urban.:** A stiff erect herb with pink, purple or white flowers seen in wet places, pasture lands, on rocks, etc.

Pollen morphology: Fresh pollen grains yellow in colour. Grains are spherical. The diameter of the pollen grain 7.14µ.

Pollen germination: 20% of pollen grains germinated in 5% sucrose solution. The tube length attained 14.28µ.

Pollen fertility: The percentage of fertility 86.35% and percentage of sterility 13.65%.

***Limnophila heterophylla*, Benth.:** Plant a rather tall, erect herb, with leaves of all three forms, the stems often from the nodes of a long under-water, horizontal stem with many capillary leaves.

Pollen morphology: Fresh pollen grains yellow in colour and spherical in shape. The size of the pollen grain 8.568µ diameter.

Pollen germination: 13% pollen grains germinated in 4% sucrose solution. The pollen tube attained a length of 17.136µ and width of 4.284µ

Pollen fertility.: The percentage of fertility 88.6% and percentage of sterility 11.4%.

***Scoparia dulcis*, Linn.:** A glabrous under shrub, sometimes up to 3 ft in height, with small white flowers and lanceolate or elliptic – lanceolate coarsely serrate leaves. Entire plant possess anti-inflammatory and diuretic properties.

Pollen morphology: Fresh pollen grains yellow in colour and spherical with a diameter of 7.14µ.

Pollen germination: Maximum germination of pollen grains was 16% in 40% sucrose solution. The pollen tube attained a length of 15.708µ and width 2.856µ

Pollen fertility: The percentage of fertility 92.5% and percentage of sterility 7.5%.

***Torenia bicolor*, Dalz.:** A small trailing and rooting herb with small leaves and the corolla with dark blue or violet upper and white lower up. Commonly called 'Kakkapu'.

Pollen morphology: Pollen grains are yellow in colour, spherical and the diameter of the grain was 9.996µ.

Pollen germination: Maximum germination was obtained in 5% sucrose solution with 15% germination. The tube length attained was 14.28µ and a width of 3.28µ

Pollen fertility: The percentage of fertility 91.5% and percentage of sterility 8.5%.

***Torenia courtallensis*, Gamble.:** A trailing herb with long decumbent stems and long pedicels, flowers bilae, the lower lip mottled with purple.

Pollen morphology: Pollen grains are yellow in colour. Grains are spherical. The diameter of grain 8.568µ.

Pollen germination: 20% of germination was observed in 4.5% sucrose solution. The length of the pollen tube attained 17.136µ and width of 2.856µ.

Pollen fertility: The percentage of fertility 88.6% and percentage of sterility 11.4%.

***Torenia parviflora*, Ham.:** A small decumbent annual up to 8 in height with blue pedicellate flowers clustered in the upper axils.

Pollen morphology: Fresh pollen grains yellow in colour and spherical and of diameter 8.568µ.

Pollen germination: 25% of germination was observed in 5% sucrose solution. The length of pollen tube was 18.564µ and width of 2.384µ.

Pollen fertility.: The percentage of fertility 90% and percentage of sterility 10%.

***Vandelia crustacea*, Benth.:** A glabrous or pubescent annual herb, branching diffusely and rooting the corolla purplish or white.

Pollen morphology: Fresh pollen grains yellow in colour. Grains spherical and its diameter 7.14µ

Pollen germination.: 20% germination was attained maximum at 5% sucrose solution. The tube grew and attained length of 17.136µ and width of 4.284µ

Pollen fertility.: The percentage of fertility 83.3% and percentage at sterility 16.7%.

***Vandelia pyxidaria*, Maxim.:** Plant is a slender divaricatingly branched herb with small apparently white flowers.

Pollen morphology: Pollen grains yellow in colour. Grains are globose measuring to about 8.568µ in diameter.

Pollen germination: 16.66% of germination was attained in 4.5% sucrose solution. The tube grew and attained a length of 17.13µ and width of 2.856µ.

Pollen fertility: The percentage of fertility 91.66% and percentage of sterility 8.33%.

The members of Acanthaceae have more of oblong shaped pollen grain, except for *Andrographi spaniculata* and *Eranthemum*

nervosum, where the pollen grains were spherical. In both, above mentioned species, the maximum percentage of germination was observed. In *Andrographis paniculata*, 32.43% of pollen germination was observed in 6% sucrose solution. Whereas, *Eranthemum nervosum*, of diameter 28.56µ, germinated to a greater extend. 32% germination was observed 5.5% sucrose solution, after 90 minutes the pollen tube attained a length of 21.42µ and 7.14µ in width. Another noteworthy observation is that, though some palynologists (Nair 1970) opined that the pollen grains of large size will not germinate in any medium, which is seen contradictory to the above.

Another inference from the present study is that the pollen grains of *Adhatodabeddomei, Andrographis paniculata, Justicia betonica, Justici aprostrata*and *Ruelli aprostrata* showed maximum germination in 6% sucrose solution with maximum germination of 30%, 32%, 52%, 14.27% & 10% respectively. Percentage of germination was comparatively less in *Justici aprostrata*and *Ruellia tuberosa.*

In C*ross andraundulaefolia, Ecbolium Linneanum* Var. *latevirens*, *Ecbolium linneanum* Var. *rotundifolium* and *Hygrophila anugustifolia*, the suitable germination medium is 5% sucrose solution and the obtained germination percentage were 25.5%, 13.33%, 15.5% & 20% respectively. The length of the pollen tubes noticed is 28.56µ, 34.27µ, 12.85µ and 15.708µ respectively.

In the members of Scrophulariaceae, all the pollen grains were spherical in shape. The pollen grain of *Toreniabicolor* measured the maximum of 9.996µwith 20% germination in 5% sucrose solution. The length of the pollen tube measured is 14.28µ and width is 3.28µ.

In *Ilysantheshys sopioides, Ilysanthes minima, Ilysanthes serrata, Scopari adulcis, Toreni aparvifloraand Vandeli acrustacea*, the pollen grains showed maximum percentage of germination in 5% sucrose solution and the obtained germination were 20%, 15%, 20%, 16%, 25% and 20% respectively. The length of pollen tube are 15.708µ, 14.28µ, 14.78µ, 15.708µ, 18.564µ and 17.136µ respectively. The pollen grains of *Limnophilaheterophylla* germinated in 4.5% sucrose solution with maximum germination of 13% with a pollen tube of length 17.136µ.In *Vandeli apyxidaria*, the favourable concentration of sucrose solution is 4%, where the pollen grains germinated 16.66%. The pollen tube length noticed in that medium was 17.13µ.

In the present study, the *in vitro* pollen germination of some selected taxa shows variation to a great extent. In case of *Andrographis paniculata*, the germination is comparatively low when compared to that of *Justici abetonica* which is the highest. *Andrographis paniculata* is a very successful species showing dominant population in the community. On the other hand, *Justicia betonica* has maximum *in vitro* germination. However, they are not widespread as *Andrographis paniculata*. The low pollen germination here is not an index of the survival of the species as several parameters aids to the widespread of the species.

Discussion: Acanthaceae members possess both oblong and spherical grains whereas in members of Scrophulariaceae the pollen grains were spherical. According to Maheshwari [1963] the diameter of the pollen tube bears no relation to the size of the pollen grains. But in *Eranthemum nervosum*, the pollen grains measured 28.56µ while the pollen tube attained a diameter of 17.136µ. In *Ecbolium linneanum* Var. *Rotundi folium*, the pollen grains measures 5.99µ in length and 19.9µ in width, while the diameter of pollen tube was 4.2µ. Even in *Toreni abicolor*, with the pollen grain measuring 9.996µ, the diameter of pollen tube measured 3.28µ. In *Ilysanthes minima*, the pollen grain measuring 5.71µ, the diameter of pollen tube measured 2.856µ. So the present study reveals that, at least in some members of Acanthaceae and Scrophulariaceae, there exists a relationship between the size of the pollen grains and the diameter of the pollen tube.

In this present study it was inferred that highest percentage of germination is in *Eranthemum nervosum*, *Toreni abicolor*, *Andrographis paniculata* and *Ilysanthes minima*. It is also interesting to note that among these four taxa *Eranthemum nervosum* and *Toreni abicolor* show the biggest pollen grains and smallest grain were observed in *Andrographis paniculata* and *Ilysanthes minima*. From this we can infer that there is no such relationship between the pollen size and germination percentage, as Nair (1970) reported.

Estimation of pollen sterility based on staining reaction is the earliest common method used, but it is not a precise method because some of the stained and apparently fertile pollen grains may not germinate both under *invitro* and *invivo* conditions. So it is suggested that the estimation of pollen fertility using sucrose medium is more effective, andcan be widely used.

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