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POLLEN MORPHOLOGY, VIABILITY, GERMINATION AND TUBE GROWTH OF SOME PLANT SPECIES

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ABSTRACT

The present investigation was to study pollen morphology, viability, germination and tube growth of some plant species in basic culture medium. The morphological observations of the studied taxa showed that most of the pollen grains are three zonicolporate, prolate or subprolate with three apertures. The results of the pollen viability revealed that the maximum staining was observed at 90 minutes at 55ºC and colour variation ranging from center of mass red, edges to pink, slightly orange, pink and red. The results of the germination and tube growth revealed the medium containing growth hormones such as IAA, 2,4-D was the most suitable for rapid pollen germination and formation of long pollen tubes than the medium treated with sucrose, boron and KCl in all the experimental plant species.

KEYWORDS: Experimental species, Pollen Morphology, Viability, Germination, Tube growth.

I. INTRODUCTION

Angiosperm pollen grains are unusual vegetative cells that contain sperm cells, cell walls and plasma membranes. The pollen morphological characters, is a useful tool in better understanding of the taxonomic disputes and classification of plants, have been critically employed in several angiosperm families since decades. It is an interpretative key for a natural classification is now debated.[1] Palynological characters are considered as a good, if not exclusive tool for the definition of a taxonomic group and a description of the pollen is considered quite necessary in the diagnosis of new species.[2] The need for assessing viability of pollen used in artificial pollination and in breeding experiments, understanding of sterility

problems and hybridization programs, fruit breeding programs and evolutionary ecology.[3,4,5,6]

In recent years, pollen germination and pollen tube development are used for determining the importance of cytoskeleton in cell growth and differentiation.[7] In some species, pollen germinates in atmospheric humidity and H_2O, some pollen germinates in basic medium containing 10% sucrose and 0.01% boric acid *in vitro*.[8,9,10] Pollen tube growth proceeds through tip extension and can be affected by many factors, including temperature, medium osmolarity and the availability of calcium, zinc and boron. During pollen tube extension, the growth direction is continuously reoriented by external signals and physical obstacles.[11,12]

In the present investigation an effort was made to study pollen morphology, viability and the effect of different concentrations of sucrose, boron, KCL and growth regulators such as IAA and 2,4-D on pollen germination and tube growth of some plant species in basic culture medium. The experimental plant species like *Cassia auriculata* (L.), *Vinca roseus* (L.), *Datura metel* (L.), *Tephrosia purpurea* (L.), *Ixora coccinea* (L.), *Solanum trilobatum* (L.), *Hibiscus rosa-sinensis* (L.) and *Tradescantia spathacea* (Sw.).

II. MATERIALS AND METHODS

Pollen Viability

The experimental plant species were collected from the garden of Botany department, Holy Cross College (Autonomous), Trichy-2. The collected fresh pollen grains were inoculated in a drop of 2, 3, 5-triphenyl tetrazolium chloride solution on a glass slide, cover with cover slip and keep the slide in a petriplate lined with moist filter paper and kept in dark for 30-60mins at 30-37^{o}C. After treatment pollen grains were observed under the microscope.

Pollen Germination and Tube Growth

The pollen grains of these flowers were surface sterilized with 0.1% mercuric chloride for 5 minutes and following by several times in distilled water. Then the pollen grains were inoculated with the Brewbaker Kwack (1963) medium containing different concentrations of sucrose and growth hormones. Simultaneously the pollen grains were inoculated in normal sugar solution (without medium) which constituted as the control. All the ontogenic and developmental stages were microphotographed using stereomicroscope fitted with Pentax camera. The calibration of size of the pollen grains were represented in scale bar as micrometer(µ). The magnification was denoted as bar (1cm represents 1µm).

Brewbaker Kwack (1963) medium[13]

S.No.	Composition of the nutrient medium	
	Constituents	**Amount/liter**
1.	Sucrose	100g
2.	H_3BO_3	100mg
3.	$Ca(NO_3)_2.4H_2O$	300mg
4.	$MgSO_4.7H_2O$	200mg
5.	KNO_3	100mg

III. RESULTS AND DISCUSSION

Pollen Morphology

Pollen morphological studies have been great importance and play significant role in distinct grouping or segregation of closely associated species. The processes like principal component analysis, principal co-ordinate analysis and cluster analysis produces hierarchical classification of entities based on similarity matrix, distance matrix and dendrogram.

The details of the morphological observations of the studied taxa for the present work are given in Table -1. The results showed that most of the pollen grains are three zonicolporate, prolate or subprolate with three apertures at equatorial region. The apertures are ranging from pantoporate, 3-colpate, 3-4-6-colpate or pantocolpate condition. The diameter of pollen ranges from 24.31µm to 87.21µm. Among the eight plant species studied the pollen grains of *H. rosa-sinensis* showed the special characteristic features which are 80 - 180µm in diameter, apolar, pantoporate, globose to spheroidal. Spines are dimorphic, longer with sharp and pointed apex and shorter with slightly obtuse apex.

Table-1: Morphology and Diameter of Pollen grains

Name of the Plant species	**Family**	**Pollen Morphology**	**Diameter of the pollen grains (µm)**
Cassia auriculata L.	Fabaceae	Spheroidal, tripolate	24.31
Vinca roseus L.	Apocynaceae	Prolate, Spheroidal, Projection absent, Tripolate	27.20
Datura metel L.	Solanaceae	Spheroidal, tripolate	37.00
Tephrosia purpurea L.	Fabaceae	Oblate-spheroidal	23.10
Ixora coccinea L.	Rubiaceae	Globose, zonoclporate, verucate	43.21
Solanum trilobatum L.	Solanaceae	Oblate, spheroal	28.33
Hibiscus rosa-sinensis L.	Malvaceae	Spheroidal, polynantoporate, tectum Granulate	80.56
Tradescantia spathacea Sw.	Commelinaceae	Oblate-spheroidal	87.21

Such studies have earlier performed in the genus *Ficus* L., *Acalypha* L., *Senna* Mill., *Indigofera* L., *Exacum* L. and *Caesalpinia* L. by taking an account of the morphological quantitative characters.[14,15,16] Malvaceae family is fairly advanced because of the echinate sculpturing and pantoporate character of its pollen grains.[17] The number, height, size, shape, position and surface distribution of the spines vary in the different plant families in which they occur and constitute some of the most significant characters for identification purposes.[18,19] External marking of the pollen grains is described as the best, most constant and distinct character by which pollen grains may be delimited at different taxonomic levels in case of stenopalynous families.[20]

Pollen Viability

The need for assessing pollen viability is essential when pollen is used in artificial pollinosis and breeding experiments. It is also an imperative approximation to understand sterility problems, evolutionary ecology and in hybridization programs. The results revealed that the aggregation of colour appears to increase with time of exposure. Table -2 showed the effect of time on staining of pollen grains with 2, 3, 5-triphenyl tetrazolium chloride at room temperature. The maximum staining was observed after 90 minutes at 55°C. The viable pollen grains showed the colour variation ranging from center of mass red, edges to pink, slightly orange, pink and red.

Most of the pollen grains used for the present study showed good percentage of viability except the pollen grains of *Hibiscus* and *Tradescantia*. The intensity of pollen staining was observed due to decrease towards the margin of the cover slip and in regions of air bubbles. The observations indicate that decrease in the availability of air bubble with the increases in coloration of the pollen. The possible reason for pollen failing to stain a uniform red may be the inhibition of reduction of TTC by air.

Table-2: Effect of time on staining of pollengrains with 2, 3, 5-triphenyl tetrazolium chloride at room temperature

Name of the Plant species	Time (minutes)	Observation	Viability percentage
Cassia auriculata L.	90	Center of mass red	79.6
Vinca roseus L.	80	Slightly red	77.6
Datura metel L.	65	Center of mass red, edges colourless to pink	69.4
Tephrosia purpurea L.	70	Slightly pink	77.6
Ixora coccinea L.	75	Center of mass red, edges	68.1

		colourless to pink	
Solanum trilobatum L.	80	Slightly pink	75.7
Hibiscus rosa-sinensis L.	60	Slightly orange	65.4
Tradescantia spathacea Sw.	50	Slightly red	77.8

These results are in agreement with several authors who showed that pollen would not stain unless a cover slip was used, or the cover slip was ringed with vaseline and simple agitation of pollen in % the dye solution also suppressed coloration.[21] Some researchers reported that TTC is strongly absorbed by lipids; also, ungerminated pine pollen is reported to contained up to 14 percent fat.[22,23,24] In seed studies a rapid and simple test of germination capacity has been developed using 2,3,5-triphenyltetrazolium chloride (TTC). This colorless dye solution is reduced by dehydrogenase enzymes to an insoluble red formazan complex in living cells.[25]

Pollen Germination

The results of germination studies revealed that lowest pollen germination was observed in control where the medium alone used along with 100mg/l of sucrose used for the present study. The lowest germination was observed in *D. metel* L. and *I. coccinea* L. The germination percentage was highest in most of the plant species studied where the medium containing growth hormone such as IAA and 2,4-D than the medium containing 200mg/l sucrose, boron and KCL (Table-3). In some plant species boron played an important role in pollen germination and tube growth. Boron can promote pollen germination and helps to grow pollen tube rapidly. So the germination and growth of pollen tube can be considerably improved through the addition of boric acid of appropriate concentrations.

Table-3: *In vitro* germination of Pollen grains in Brewbaker Kwack medium

Name of the Plant species	**Germination Percentage**		
	Control	**Medium with sucrose, boron and KCL**	**Medium with IAA and 2,4-D**
Cassia auriculata L.	15	46	84
Vinca roseus L.	12	38	71
Datura metel L.	12	27	46
Tephrosia purpurea L.	18	48	67
Ixora coccinea L.	13	36	48
Solanum trilobatum L.	11	28	56
Hibiscus rosa-sinensis L.	10	23	73
Tradescantia spathacea Sw.	14	29	68

The investigations have examined for the effects of boron on changes in the chemical components of pollen tubes in coniferous species. In general, compared with angiosperm pollen tubes, coniferous pollen tubes grow slowly and do not form callose plugs that they differ in wall deposition and construction.[26] As there was no or little germination of pollen in control without sucrose and growth hormones in an experiment and lower concentrations of sucrose in the culture medium decrease the supply of carbon available to the culture, being the osmotic potential means changed, may inhibit, the formation of pollen germination and pollen tubes *in vitro*.[27] Germination in *Sorghum bicolor*, *Datura metel* and *Abelmoschus esculentus* with 1-3ppm GA_3 showed maximum pollen germination.[28,29,30] Except ABA all other growth regulators (IAA, GA_3, Kn and Ethylene) increased both germination and pollen tube growth at all the concentration used in *Cicer arietinum*.[31]

Pollen Tube Growth

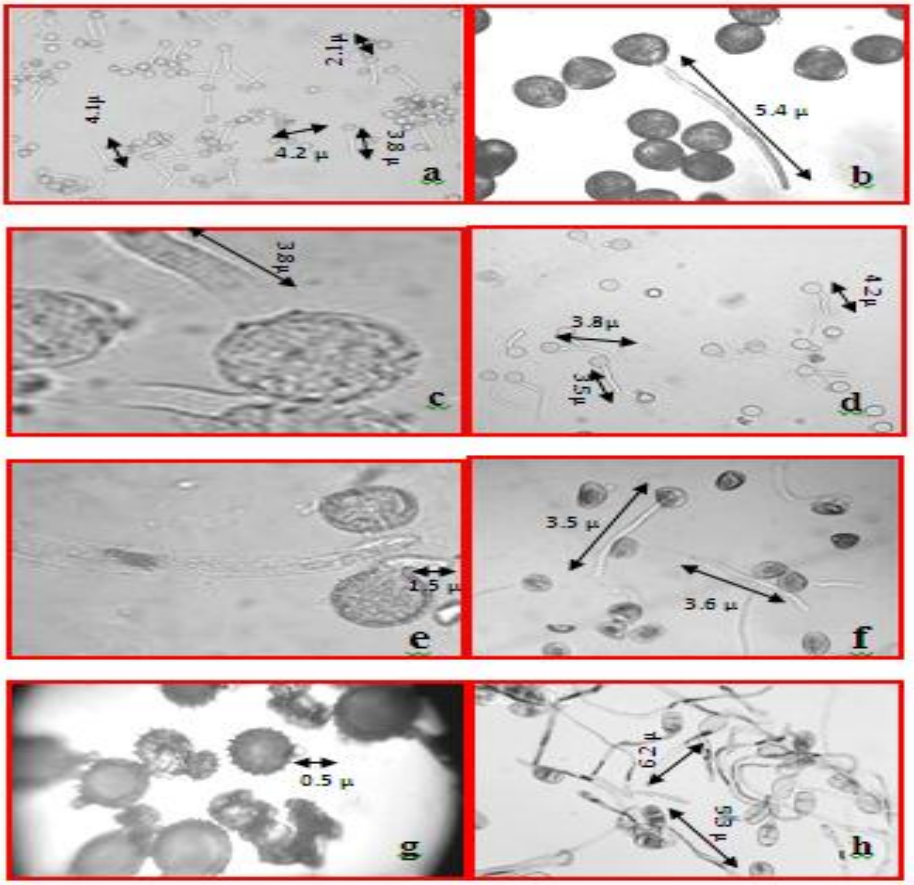
The pollen tube formed in many species is a massive structure relative to the reserve materials stored in the pollen grain. These nutrient reserves are lipids, starch and sugars. The results of the pollen tube growth revealed that more number of pollen tube was observed in the medium containing 200mg/l of sucrose along with boron, KCL, IAA and 2,4-D in most of the plant species studied. Table-4 showed the formation of longest pollen tube was observed in *T. spathacea*, *V. roseus* and *C. auriculata* which were 91.76, 79.92 and 74µm respectively. The medium treated with growth hormones such as IAA and 2,4-D alone was the most suitable for rapid pollen germination and formation of long pollen tubes, in addition to existing the balance of osmotic solution and providing energy for the tube growth (Plate-1).

Table-4: Effect of medium with sucrose, Boron, KCL, IAA and 2, 4-D on length and breadth of the pollen tube

Name of the Plant species	Pollen Tube Growth		
	Effect of medium	Length (µm)	Breadth (µm)
Cassia auriculata L.	Sucrose +Boron+KCL+IAA	74.00±26.13	17.76±11.20
Vinca roseus L.	Sucrose +IAA	79.92±34.20	34.04±16.34
Datura metel L.	Sucrose +Boron	56.24±21.70	26.64±14.27
Tephrosia purpurea L.	Sucrose +Boron+2,4-D	62.16±24.32	11.84±9.80
Ixora coccinea L.	Sucrose +Boron+KCL	22.20±11.30	16.28±7.34
Solanum trilobatum L.	Sucrose +2,4-D	51.80±17.62	28.12±13.67
Hibiscus rosa-sinensis L.	Sucrose +IAA	7.40±0.80	13.30±28.90
Tradescantia spathacea Sw.	Sucrose +Boron+2,4-D	91.76±42.75	31.08 ±17.43

Several studies have been examined for the impact of boron on development of reproductive organs. Because the pollen tubes represent a fast growing system and are sensitive to boron deficiency and the morphological effects of boron during pollen tube growth in angiosperms have also been investigated.[32] Stimulation of pollen tube growth by IAA, Kinetin, and GA_3 is reported in *Calotropis*, *Annona* and *Arachis*.[33,34,35]

PLATE-1 POLLEN TUBE GROWTH



a.	*Cassia auriculata L.*
b.	*Vinca roseus L.*
c.	*Datura metel L.*
d.	*Tephrosia purpurea L.*
e.	*Ixora coccinea L.*
f.	*Solanum trilobatum L.*
g.	*Hibiscus rosa-sinensis L.*
h.	*Tradescantia spathacea Sw.*

IV. CONCLUSION

In the present investigation an effort was made to study pollen morphology, viability, germination and tube growth in contributing to the knowledge of the reproductive biology

and subsidizing their conservation, management and utilization. Although it was a preliminary laboratory study, it provided encouraging results and basis for future research.

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