RESEARCH ARTICLE

VARIATION IN RATE OF POLLEN FERTILITY IN SOME MEMBERS OF FAMILY RUBIACEAE

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ABSTRACT

Aerobiology is a scientific and multidisciplinary approach focus on the transport of organisms and biologically significant materials (Edmonds 1973). It deals with the study of air spora such as algal filament, fungal, bryophyte and pteridophyte spores and pollen grains of gymnosperms and angiosperms and other microorganisms (S. T. Tilak 1982). Since air currents play significant role in the dispersal and pollination of pollen grains, the palynological studies can be interestingly associated with aerobiology. The pollen grain which is a highly reduced male gametophyte in flowering plants is one of the efficient research tools for studying the effect of various chemicals on its metabolism since it carries the male genetic material. Sucrose is reported as the best source of carbohydrate for pollen germination and pollen tube growth [Adams (1916), Brink (1924), Hrebetova and Tupy (1964)]. The Rubiaceae family is world's fourth largest flowering plant family with around 11,000 species, including coffee (coffea), quinine (cinchona), and beautiful tropical ornamentals such as *Gardenia.* Around 400 species of this family have become rare and endangered. Therefore, the members of Rubiaceae family are an important collection for conservation, research, horticulture and education. In the present study, three members of Rubiaceae family were used as experimental models to study their pollen physiology. The pollen grains of plant species, *Hamelia patens, Mussaenda sps., Gardenia thunbergia* , were studied for pollen germination and pollen tube growth. The pollen grains of the above plant species were treated with 10%, 15% and 20% sucrose concentrations. Effect of Brewbaker and Kwack's medium (Brewbaker and Kwack, 1963) on the pollen grains of above species was also examined. *Hamelia patens* showed best results followed by *Mussaenda sps* followed by *Gardenia thunbergia*. Interestingly both *Hamelia patens* and *Gardenia thunbergia* showed best efficiency in 20% sucrose concentration while *Mussaenda sps* was most efficient in 10% sucrose concentration. The results obtained lead to the conclusion that Brewbaker and Kwack's medium gives more satisfactory results as compared to the results obtained using only sucrose as a growth medium.

Keywords : Palynology, *Hamelia patens, Mussaenda sps., Gardenia thunbergia,* Brewbaker and Kwack's medium, Rate of fertility

INTRODUCTION

Aerobiology is a scientific and multidisciplinary approach focused on the transport of organisms. Since air currents play significant role in the dispersal and pollination of pollen grains, the palynological studies can be interestingly associated with aerobiology.

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The pollen grain is a highly reduced male gametophyte in flowering plants, generally shed off from plants after the dehiscence of the anther which carries the male genetic components transmitted in sexual reproduction of all higher plants and thus, can be used as a very efficient research tool for studying the effects of various chemicals on its metabolism. Maheshwari, (1963) and se veral other scientists demonstrated the role of pollen in sexual reproduction. Besides embryologists and morphologists, pollen grains have attracted the attention of physiologists, bio-chemists, cell biologists,

geneticists and to a large extent the plants breeder. The pollen grains are also being effectively used in genetic engineering. The use of pollen grain has already been extended in many more areas of research.

Rubiaceae family is world's fourth largest flowering plant group with around 11,000 species. Around 400 species of this family have become rare and endangered. Therefore, the members of Rubiaceae family are an important source for conservation, research, horticulture and education. In the present study, three members of Rubiaceae family were used as experimental models to study their pollen physiology. The pollen grains of plant species, *Hamelia patens, Mussaenda sps.* and *Gardenia thunbergia* were studied for pollen germination and pollen tube growth. *Hamelia patens* is a dependable and useful perennial shrub with a long blooming season. It is extremely drought tolerant and thrives in any soil as long as it is well- drained. The stems and leaves have been used for tanning and a concoction reportedly is used for various medicinal purposes. It is also rich in active phytochemicals such as alkaloids and flavonoids. Propagation is done by seed and half-ripen fruit cuttings. *Hamelia patens* is a heat tolerant species that has potential value as a resource efficient landscape plant. *Mussaenda* is an ornamental shrub suited to tropical and subtropical climates with a bright future both as landscape plants and in floral decorations. The most distinctive feature of *Mussaenda* is that the floral display is primarily derived from the calyx. *Mussaenda* seed is significant for hybridization while vegetative methods are used for commercial propagation. Alternative means of propagation include grafting and layering. *Gardenia* is an evergreen perennial shrub with medium growth rate showing whorled phyllotaxy. The waxy, highly fragrant white flowers of *Gardenia* have great horticultural potential due to its strong aroma and aesthetic value. Large species number and the great morphological, anatomical and biological variation make Rubiaceae a very interesting family for study. Considering the economic importance and horticultural significance of family Rubiaceae, the above mentioned three members of the same family were considered for the study. In the present work, the comparative account of the above three plant species is done with respect to the variation in their rate of pollen fertility

MATERIAL AND METHODS

Two distinct parameters: pollen germination and pollen tube growth was studied from the dissected anthers of three members, *Hamelia patens, Mussaenda sps. and Gardenia thunbergia* of family Rubiaceae using acetocarmine as a stain. Since sucrose has already been concluded as the best energy supplier for pollen germination and pollen tube growth, it was taken as a basic medium for germination pollen grains (Tupy 1960); Hrebetova and Tupy (1964). 10%, 15% & 20% sucrose concentration was taken for the preparation of basic growth medium for pollen to find the optimum concentration required for pollen fertility rate. Most efficient concentration of sucrose as a basic culture medium was noted at *H. patens* (20%), *Mussaenda sps.* (10%) and *Gardenia thunbergia* (20%) respectively. After standardization Brewbaker and Kwack's medium (Brewbaker and Kwack, 1963) was used separately used for the three plants with their respective optimized sucrose solution. Pollen grains were counted randomly at 40x magnification to calculate the percentage of pollen germination. After calibrating the ocular micrometer, the pollen tubes were also measured at the same magnification.

Composition of Brewbaker and Kwack's medium is as follows:

Content	Composition of medium (mg/l)
Sucrose	100,000 (10%)
H_3BO_3	100 (0.01%)
$Ca(NO_3)_2.2H_2O$	300 (0.03%)
$MgSO_4.7\ H_2O$	200 (0.02%)
pH	7.3

The observations clearly indicated better results with Brewbaker and Kwack's medium as compared to only sucrose as growth medium.

RESULTS & DISCUSSION:

Interestingly both *Hamelia patens* and *Gardenia thunbergia* showed best efficiency in 20% sucrose concentration in both the parameters (rate of pollen germination and rate of pollen tube growth) while in *Mussaenda sps* best positive result in both the parameters was observed in 10% sucrose concentration. Highest germination in *Hamelia patens* was recorded as 55.5%(in only sucrose) and 66.66%(in BK media), whereas pollen germination in *Gardenia* was found to be 31.25% (in only Sucrose) and 38.45% (in BK medium) in the same concentration. Highest pollen germination recorded in *Mussaenda sps* was 57.14% (in sucrose only) and 60% (in BK medium). Highest pollen tube length observed in *Hamelia patens* was 168.75 µ (in only sucrose) and 281.25µ (with BK media) whereas in *Gardenia thunbergia* maximum pollen tube length was found to be 71.25µ (in only sucrose) and 86.25µ (in BK medium). In *Mussaenda sps*. maximum pollen tube length noted was 116.25µ (in only sucrose) and 157.5µ (in BK medium). Incidentally in *Gardenia thunbergia*; neither pollen germination nor growth of pollen tube was noticed in Brewbaker and Kwack's medium with standardize 10% sucrose concentration. However minimum efficacy in both parameters was observed in *Gardenia thunbergia* as compared to both *Hamelia patens* and *Mussaenda sps.*

Sucrose is the best source of carbohydrate for pollen germination and pollen tube growth (Tupy 1960); Hrebetova and Tupy (1964). Thus significant tube growth in adequate sugar solution, particularly in Sucrose have been reported by Brink (1924); and Gollmick (1942). Highest rate of germination and better tube growth was observed on sucrose medium as compared to other sugar solutions i.e. glucose and fructose, which indicates the higher rate of respiration of a particular plant (Hrabetova and Tupy (1961). The germination of pollen of cabbage was maximum in sucrose, while longest pollen tube was observed in raffinose (Chiang 1974). Hrabetova and Tupy (1961,1964) have suggested that in some species pollen germination and tube growth showed considerable increase with many other sugars as well. Balasubrahmanyan (1959) found that 6% sucrose was the best medium for guava pollen germination. The germination of pollen of apple was observed in 10% sucrose Tupy (1960). Arromov (1956) noticed best germination of grape pollen in 10 – 20% sucrose medium. Chiteley and Naik (1971) noticed 20% sucrose as best medium for germination in *Sesbania grandiflora*. *Dolichos lablab* showed 29% germination in 25% sucrose. The presence of sucrose, glucose and fructose in pollen was also reported by O'Kelly (1955), Kessler (1960); which are utilized in respiration. Repeated studies on extracts of pollen by researchers have revealed the presence of endogenous sucrose in the pollen. It has been also noticed that 2 celled pollen grains need 10-20% sucrose, while 3 celled pollen need as high as 50% for germination. Various types of sugars were used from time to time by many researchers (Bishop (1949) - Lactose).

Table 1. Pollen germination

Plant name	Sucrose conc.	Basic Sucrose medium	Brewbaker & Kwack's medium
Hamelia patens	10%	37.5%	47.05%
	15%	42.85%	54.16%
	20%	55.5%	66.66%
Mussaenda sps.	10%	57.14%	60%
	15%	42.85%	43.75%
	20%	16.66%	26.66%
Gardenia thunbergia	10%	--	--
	15%	12.5%	16.5%
	20%	31.25%	38.45%

Table 2. Pollen tube growth (in µ)

Plant name	Sucrose Conc.	Basic Sucrose medium	Brewbaker & Kwack's medium
Hamelia patens	10%	75	93.75
	15%	108.75	131.25
	20%	168.75	281.25
Mussaenda sps.	10%	116.25	157.5
	15%	78.75	78.75
	20%	56.25	71.25
Gardenia thunbergia	10%	--	--
	15%	67.5	71.25
	20%	71.25	86.25

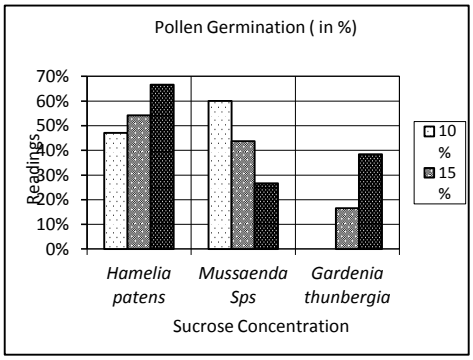

Fig 1. Pollen germination

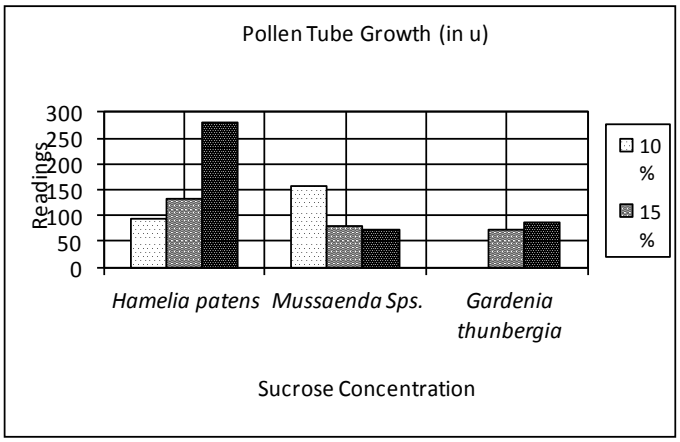

Fig 2. Pollen tube growth

O'Kelley, (1955) have also studied the pollen germination in many plants. When pollen of *Benincosa hispida* and *Lagenaria vulgaris* were supplemented with dextrose and galactose, Vasil (1960) demonstrated that pollen germination was poor. On studying the effect of glucose, sucrose and fructose on apple pollen, Vasil (1960) reported better germination in sucrose.

All the above mentioned reports have clearly suggested maximum potential of sucrose as a carbohydrate source for energy. Interestingly, it was observed that amongst all the three cultivars, *Hamelia patens* showed best result in both pollen germination as well as pollen tube length formation. Therefore it was found that the results were substaintially favourable in *Hamelia patens* in contrast to the *Mussaenda sps* and *Gardenia thunbergia.*

Pollen grains are very specialized and complex plant cells. Thus pollen germination and growth of pollen tube are important research materials for morphological, physiology, biotechnological, ecological, evolutional, biochemical, molecular and biological studies [Dane, F., Olgun, G. and Ozlem, D. (2004)]. During *in vitro* pollen germination and tube growth not only the effect of enzyme activity but also the effect of boron, calcium, light, hormones and other factors were studied for different plants [Prajapati and Jain, (2010)]. Satisfactory pollen germination requires sugar, especially sucrose with other substances [Patel, V.A., Patel, D. and. Jain, B.K. (1997)]. In pollen tube culture, externally supplied sugars play a vital role in controlling osmotic pressure of the medium and simultaneously increase the growth rate of pollen tubes [Baloch, M.J., Lakho, A.R., Bhutto, H. and Solangi, M.Y. (2001)]. It was found that boric acid has pronounced stimulatory effect on germination and pollen tube growth [Taylor, L.P.and Hepler, P.K. (1997) and Feijó, J.A., Malho, R. and Obermeyer, G. (1995)]. Ca^{2+} plays a key role in the regulation of pollen tube growth [Steer, M.W. and Steer, J.M. (1989)]. According to Bendnarska, K. (1989), calcium is an inorganic substance with notable effect on pollen tube growth and an essential requirement of pollen tube growth. Dickinson, D.B. (1967) has also reported that calcium controls the permeability of pollen tube membrane. Thus supplementation of calcium in the medium leads to development of straight and rigid pollen tube with vigorous growth. A positive correlation between rate of pollen tube growth and quality of the resulting progeny was also explained [Delph-Lynda, F., Weining, C. and Suttivan, K. (1998)]. According to Brewbaker and Kwack [Brewbaker, J. L. and Kwack, B.H. (1963)] magnesium ions enhance the effect of calcium ions resulting in vigorous growth of pollen tube.

In the present study, it was also observed that the results obtained with Brewbaker and Kwack's medium showed more satisfactory results as compared to the results obtained using only sucrose as a growth medium.

CONCLUSION:

Considering overall results, it can be concluded that pollen physiology with respect to pollen germination and pollen tube length is showing marked diversity in all the three members. Since the germination of pollen is directly responsible for next generation of the plant, *Hamelia patens* showing best performance should be extensively cultivated. Simultaneously, it has also been noticed that *Hamelia patens* showed longer tube length in pollen which corroborate the probable answer to vigorous rate of the resultant progeny in this member. Since *Hamelia patens* is of great horticultural significance as well as in herbal and various other industries it can be concluded that the member *Hamelia patens* can be an important source in enhancing the economy of the country.

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