

# FLOWERING AND SEED SET IN *DENDROCALAMUS GIGANTEUS* Wall. Ex Munro FROM KERALA

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## Abstract

The flowering behaviour is peculiar to bamboos, being monocarpic with most species flowering profusely at fixed intervals of several years, following which they perish. The flowering in *Dendrocalamus giganteus* is said to be infrequent with long intermast period. In spite of gregarious flowering seed set is reported to be rare or in limited quantity. The reason for poor seed set is chiefly developmental in nature. Meiosis showing regular segregation producing viable gametes and acceptable level of pollen viability and stigma receptivity, the only impediment for successful fertilization is the pronounced protogyny prevalent in *D. giganteus*. Further, abscission or abortion of fertilized ovule contributes to the low amount of seed set experienced in the species.

Index terms: Bamboos, *Dendrocalamus giganteus*, flowering, seed set, pollen viability.

## INTRODUCTION

Bamboos generally prevail in all Tropical and Sub-Tropical regions of the world, especially Asia and South America, some in tropical Africa and Australia. In India there are about 130 species of Bamboo (Mandal and Subramanian, 1992), more than 50 % of which are distributed in the North East.

The flowering behaviour is peculiar to bamboos, being monocarpic with most species flowering profusely at fixed intervals of several years, following which they perish. Successful flowering and associated post pollination phenomena ensures the establishment of set of future of the species. Any impediment in the process will lead to failure of seed set and survival of the species as a whole. Thus, flowering is the most important event in the life cycle of a plant. Flowering in certain species of bamboo is intriguing and seed set remains an enigma. Bamboos in general are wind pollinated and set seeds in large quantity and perishes. The seeds germinate and ensure the set future.

*Dendrocalamus giganteus* commonly known as giant bamboo belonging to the subfamily bambusoideae within the family Poaceae (Mc Clure, 1966). Distributed in the tropical and sub-tropical regions is a dense clumping species native to South East Asia and is woody growing up to 25 to 30 m tall. The culms are erect ranging in diameter 20-30cms.

Most bamboo flowers infrequently and it have been reported that flowering cycle for this giant bamboo and seed-setting can be at every 29, 40, 65, 80 years of interval. Precise interval period for the giant bamboo to flower could not be ascertained, thus the exact interval is believed to vary due to growing conditions. It was also observed that the clump that has undergone flowering did not always die as in other types of bamboos. The species is reported to have a life cycle of 75 years by Janzen (1976) while, Seethalakshmi *et. al.* reported 65 years before flowering. The species is characterized by gregarious flowering while, seed set is reported to be rare or is limited in quantity. Jalil and Shukla (2010) while studying the flowering behaviour and seed characteristics of *D. giganteus* observed that fruit/ seed setting was very poor. The average seed weight per clump was  $55.43 \pm 6.21$ mg. Ramanayake and Yakandawala (1998) observed that seed set was rare and that vegetative growth was related to seasonal rainfall, while flowering did not appear to be related to any external factors. Seethalakshmi *et. al.* (2010) reported flowering in 2007 from (Kottayam, Kerala, India, resulted in limited quantity of seed set. While seed set was absent in population flowered in Kozhikode, Kerala, India.

Presently an attempt is being made to study the reasons for poor or rare seed set in *D. giganteus* (Fig.1a, b). The pseudospikelets (Fig.1c) which are lanceolate and laterally compressed being 2 to 2.5 cms long and 0.2 to 0.5 cms width. Four to eight florets breaking up at maturity. Fertile florets increasing in size upwards (Fig.1d). Lemma 1 to 1.5 cms long and acute at the apex. Palea two keeled. Anthers six in numbers, one cm long and single stigma (Fig.1e). Fruit is caryopsis 0.5 to 1 cm long (Fig. 1f). Also, detailed cytological (meiotic) study is very much lacking, apparently owing to their long intermast period.

## MATERIALS AND METHODS

The plant materials required for the study (Floral parts and root tips) were gathered from flowering offsets of *D.giganteus* collected from Napier museum, Thiruvananthapuram and maintained in the bambusetum of Tropical Botanic Garden and Research Institute, Palode.

Cytological studies were carried out in pollen mother cells and root tip cells. Root tips were subjected to a pre-fixation treatment of 0.02M aqueous solution of 8-hydroxyquinoline for three hours at 4°C. Both root tips and young spikelets were fixed in Carnoy's fluid. Chromosome preparations were made by aceto-orcein smear and squash technique and the same were photographed using Olympus BH-2 research microscope. Mature spikelets with florets at the time of anthesis were collected and stored in glacial acetic acid for pollen morphological study. Acetolysis was done following (Erdtman, 1952). Pollen size classes were determined according to (Walker and Doyle, 1975)

To study the extent of pollen sterility, mature pollen grains were stained in 1: 1 mixture of glycerine and 2 % acetocarmine and examined under the microscope, those which stained were considered fertile and the unstained as sterile.

Pollen viability was ascertained using (1) Tetrazolium test (Hauser and Morrison, 1964; and Stanely and Linsken, 1974) and (2) Fluorochromatic reaction test (Heslop-Harrison and Heslop-Harrison, 1970). Freshly collected mature pollen grains were dusted on a drop of 0.5% TTC (2,3,5 Triphenyl Tetrazolium Chloride) in sucrose solution and incubated in humidity chamber for 30 minutes, after which they were observed under microscope and pollen grains which stained red were scored as viable. For fluorochromatic test freshly collected mature pollen grains were suspended in a drop of Sucrose-Fluorescein di Acetate mixture and incubated in a humidity chamber for ten minutes and observed under the fluorescence microscope. The pollen grains which fluoresce brightly were scored as viable.

*In-vitro* germination studies using freshly collected pollen grains were carried out in standard Brewbaker and Kwack's (Brewbaker and Kwack, 1963) medium supplemented with 1%, 2.5%, 5%, 10%, and 20% sucrose and scored for percentage of germination.

*In-vivo* germination studies were carried out in field grown plants by controlled self pollination. The florets were self pollinated and bagged. The pistils thus pollinated were carefully dissected out after the intervals of 3h, 24h, 48h and 72 hours and fixed in Carnoy's fluid for 48 hours. They were then transferred to Lactophenol solution added with a few drops of 1% cotton blue stain and incubated at 60°C for 30 minutes. The stained pistils were mounted in glycerine and observed under microscope for pollen germination and pollen tube growth.

## RESULTS AND DISCUSSION

Cytological investigation so far revealed the existence of triploids, tetraploids, pentaploids, hexaploids and aneuploids members (Richaria and Kotwal, 1940; Parthasarathy, 1946; Darlington and Wylie, 1955; Janaki Ammal 1955; Sharma and Mehara, 1972, 1975; Ghoral and Sharma, 1980; Lalithakumari, 1983). Root tip cells at metaphase revealed  $2n = 72$  chromosomes (Fig.2f). This is in agreement with earlier report (Janaki Ammal, 1945). The chromosomes were of small size and ranged from  $3.02 \mu\text{m}$  to  $1.55 \mu\text{m}$  in length. However, detailed karyomorphology was not attempted owing to small size of the chromosomes. Over 150 florets were subjected to meiotic studies. Most of the pollen mother cells did not reveal any recognizable stages. However, the stages showed normal segregation with 36 bivalents at meiotic metaphase (Fig.2d)

The pollen grains belong to the globose shape ranging in size from 28.60  $\mu\text{m}$  to 16.30  $\mu\text{m}$ . Degree of pollen sterility as revealed by acetocarmine staining is  $90.5 \pm 0.052\%$ , while that revealed by TTC is  $84.42 \pm 0.034\%$ . Fluorochromatic reaction showed  $32 \pm 0.042\%$  of the pollen to viable (Fig.2a). However, *in-vitro* germination studies in Brewbaker and Kwack's medium supplemented with 5% sucrose yielded  $18 \pm 0.039\%$  of the pollen to be fertile in terms of pollen germination (Fig.2b). No germination was recorded at sucrose concentrations below 2% and above 10%. Seethalakshmi *et. al.* (2010) reported low pollen viability of 10-15% by acetocarmine staining. *In-vivo* studies were carried out following manual pollination which was conducted following two methods. In the first, the stigmas were pollinated just as they were emerging out (Fig.2e) and in the second pollination were done after the stigmas fully emerged out (Fig.2c)

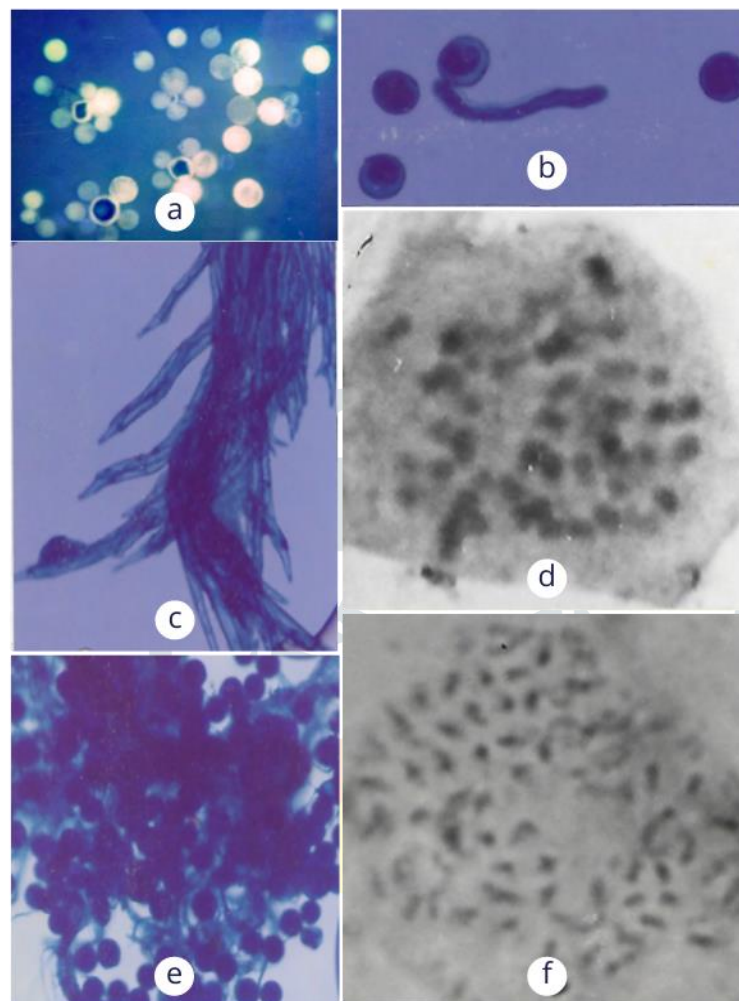


**Figure 1. a-f. *Dendrocalamus Giganteus***  
**a.**Vegetative Phase **b.** Flowering phase **c.** Florets **d.** Gynoecium Emergence  
**e.** Stamen Emergence **f.** Seedling Emergence

The longevity of pollen grains in terms of germination potential, since dehiscence of anther is found within the first 15 minutes followed by sharp decline (Fig.2f). The process of style emergence takes 12 hours to 18 hours. The process of anther emergence was completed in 6 hours.

*D. giganteus* is a clear case of protogyny (Fig.1d) and the duration of protogyny was 72 hours. The period of protogyny was pronounced in dry season (72% RH, 32°C) than during wet days (95% RH, 27.5°C). Abscission of successfully fertilized florets was observed in majority of the cases. Most of them were found to abscise between 14<sup>th</sup> and 15<sup>th</sup> day following successful selfing. Regular segregation of meiotic chromosomes account for the fair percentage of pollen germination.

The stigma seem to be receptive just as they emerge, for it is interesting to note that pollen grain were aggregated on the stigma when they were pollinated just as they emerge and showed abundant germination (Fig.2e). However, in the second instance no pollen was found on the stigmatic surface, when they were pollinated after complete extrusion of the stigma (Fig.2c). From this it may be inferred that the stigma is receptive just as they emerge and tend to lose their receptivity when they are fully emerged.



**Figure 2. a-f. *Dendrocalamus Giganteus***  
**a.** Fluorochromatic reaction test showing viable/non viable pollen x 200  
**b.** Pollen viability and germination x 400 **c.** Non adherence of pollen on stigmatic surface when pollinated after complete extrusion of stigma x 200 **d.** Regular segregation of meiotic chromosomes x 3000 **e.** Abundance of pollen adhered to stigma when pollinated just as stigma emerges x 200 **f.** Somatic chromosome at metaphase x 2000

Further, the availability of pollen in natural conditions when the stigma is just receptive (i.e., just as they emerge) is doubtful owing to the pronounced protogyny. In addition the longevity of pollen is also short (15 minutes from the time of emergence). Therefore, the short lived pollen is a limiting factor as far as pollinating a fresh set of emerging stigma if any. As a consequence the chance of successful pollination/fertilization is minimum. This could be one of the reasons for the low percentage of seed set found in this species. Moreover, even when fertilization occurred, the fertilized florets were found to abscise before seed formation. This also adversely affects the percentage of seed set. Also, reports of some clump setting seed even though in limited quantity following flowering while failure of seed set in geographically distantly located clump. Ramanayake and Yakandawala (1998), Seethalakshmi *et. al.* (2010) and Jalil and Shukla (2010) should be pondered for environmental triggers.

## CONCLUSION

*D. giganteus*, an allopolyploid with  $2n=72$  based on  $x=12$  shows regular segregation of chromosomes at meiosis, leading to the production of viable gametes and formation of seeds. However, the low percentage of seed set could be attributed to pronounced protogyny, short duration of receptivity of stigma, short lived pollen grains (viability) and abscission of fertilized ovule (florets) or abortion. Thus, the reason seems to be more of developmental in nature.

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