Studies on the absence of seed set in *Bambusa vulgaris*

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No seed set is reported in the common bamboo, *Bambusa vulgaris*, (Bambusoideae: Poaceae) so far. The causes for its sterility were studied through investigations on pollen fertility, viability, *in vitro* and *in vivo* germination and cytology. Very low pollen viability and high pollen sterility are due to meiotic irregularities. Although a small proportion of pollen grains germinated following manual pollination, pollen tubes were inhibited in the stigmatic papillae. Lack of seed set in this species seems to be the result of cumulative effect of a number of physical and physiological factors.

THE infrequent flowering and lack of sexual reproduction (seed set) in *Bambusa vulgaris*[1], one of the most commonly cultivated bamboos belonging to the subfamily Bambusoideae of Poaceae, have been a matter of curiosity among bamboo specialists[2–7]. As many as 20 incidences of flowering of this species were reported from 10 countries during the past one and a half centuries[8]. Strangely enough, no fruit set was reported from anywhere. Many earlier investigators, including McClure[2], were baffled by this unusual behaviour of *B. vulgaris*. Whereas Banik[5], and John and Nadgauda[7], attribute lack of seed set to high percentage of pollen sterility and non-viability, Koshy and Pushpagadan[6] point to the physical factors such as the inherent 'unhealthy' nature of stigma to receive pollen, the peculiar opening and closing of florets and the possible role of bristle-like hairs on palea, as barriers preventing pollination. The present investigation is therefore an attempt to study further the factors responsible for the failure of seed set in this economically important species through studies on pollen sterility, viability, *in vitro* and *in vivo* germination, in addition to cytology.

The plant materials (floral parts and root tips) were gathered from flowering offsets of *B. vulgaris*, collected from Cherthala in Alappuzha district, Kerala[6] and grown in the bambusetum of Tropical Botanic Garden and Research Institute (TBGRI), Palode, Thiruvananthapuram. Cytological studies were carried out in pollen mother cells and root tip cells. Root tips were subjected to a pre-fixation treatment in 0.002 M aqueous solution of 8-hydroxyquinoline for 3 h 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 glacialacetic acid for pollen morphological study. Acetolysis was done following Erdtman[9]. Pollen size classes were determined according to Walker and Doyle[10].

To study the extent of pollen sterility, mature pollen grains were stained in 1 : 1 mixture of glycerine and 2% acetocarmine and examined under a microscope. Those which stained were considered fertile and the unstained as sterile. Pollen viability was assessed using (1) tetrazolium test[11,12] and (2) fluorochromatic reaction test[13]. Freshly collected mature pollen grains were dusted on a drop of 0.5% TTC (2,3,5 triphenyltetrazolium chloride) in sucrose solution and incubated in a humidity chamber at room temperature in dark for 30 min. Then they were observed under the 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 diacetate mixture and incubated in a humidity chamber for 10 min 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[14] medium, supplemented with 1, 2.5, 5, 10 and 20% sucrose and scored for percentage germination.

Since there is no evidence of natural pollination[8] in this species, *in vivo* germination studies were carried out on 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 3, 24, 48 and 72 h and fixed in Carnoy's fluid for 48 h. They were then transferred to lactophenol solution added with a few drops of 1% cotton blue stain and incubated at 60°C for 30 min. The stained pistils were mounted in glycerine and observed under the microscope for pollen germination and pollen tube growth.

Root-tip cells showed variations in their chromosome constitution which ranged from $2n = 72$ to 82 (Figure 1 *b*) with still low numbers ($2n = 32$, 34). The model number is $2n = 72$, which is in conformity with an earlier report[15]. The length of the chromosomes ranged from 4.01 µm to 1.60 µm. Karyotype analysis was not possible due to indistinct centromeric position in many chromosomes. Meiotic study was carried out in 30 pollen mother cells with sufficiently clear metaphase (Tables 1 and 2). Meiosis was very irregular because of association of chromosomes into multivalents in various frequencies at diakinesis and metaphase-I (Figure 1 *a*, *d*, *e*, *f*). Although a very large number of pollen mother cells were examined, sufficiently clear meiotic preparation was evident only in about 30 cells, which also exhibited chromosome associations in various types and

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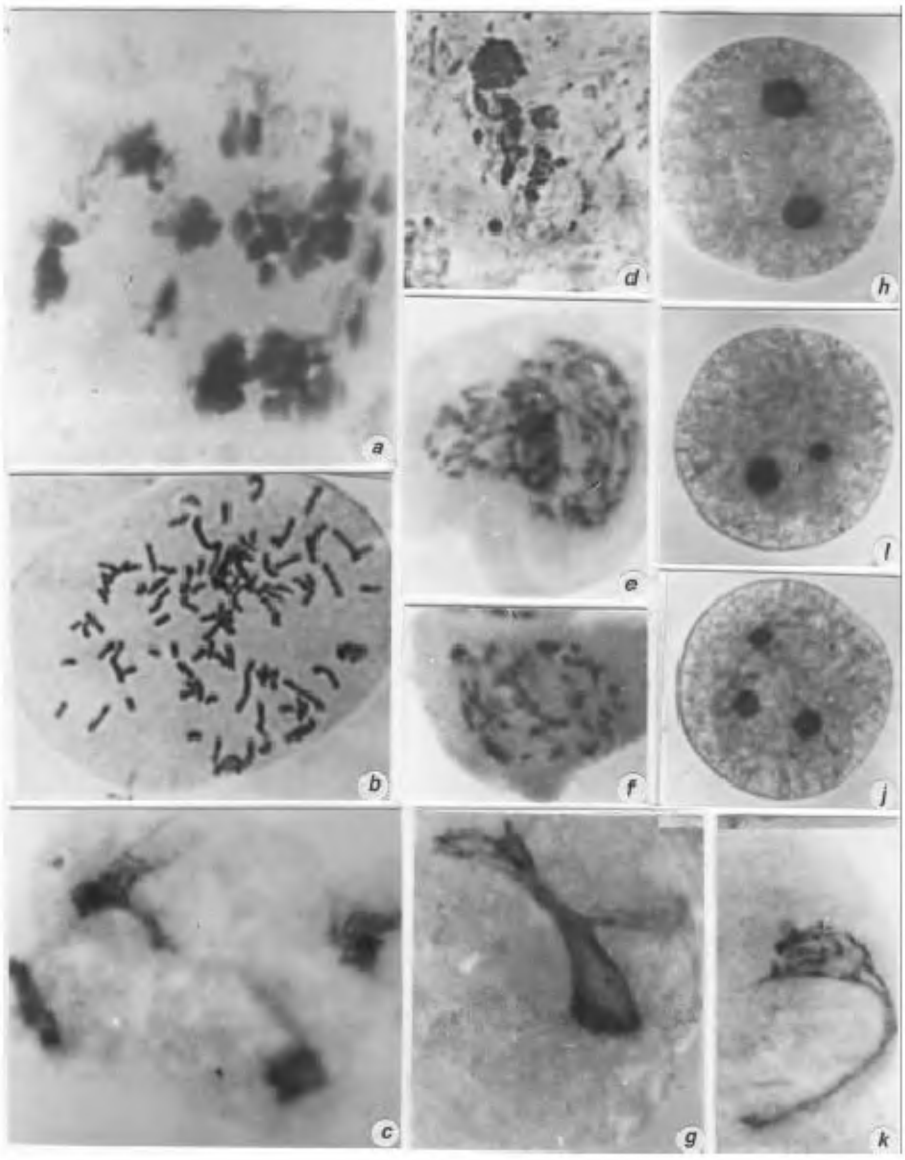

Figure 1. ***a–k***, *Bambusa vulgaris,* cytology: ***a***, Chromosome association at meiotic metaphase (quadrivalents, trivalents, bivalents and univalents), × 3000; ***b***, Somatic chromosome at metaphase showing $2n = 82$, × 1800; ***c***, Unequal distribution of chromatic material, × 2000; ***d***, Chromosome association at meiotic metaphase, × 1400; ***e***, Pre-diakinesis stage of meiotic chromosome, × 3000; ***f***, Pre-metaphase stage, × 3000; ***g***, Non-disjunction, × 1800; ***h–j***, Microspores with varying number of nuclei, × 1700; ***k***, Unipolar movement of chromosome involving laggards, × 1600.

degrees. Almost all such cells showed at least six quadrivalents and the rest trivalents, bivalents and univalents. Anaphase-I showed high degree of chromosome number variations in segregating groups (Figure 1 *c*). Owing to irregular anaphase separation, microspores with differently-sized nuclei were encountered (Figure 1 *h–j*). Lagging of chromosomes (Figure 1 *g*, *k*) was a frequent feature at anaphase.

The pollen grains of *B. vulgaris* are globose, ranging from 33.30 to 13.30 µm. They include large grains – 63%, medium grains – 24% and small grains – 13%. They possess mono-ulcerate aperture and psilate ornamentation.

The degree of sterility, as revealed by acetocarmine staining, was $71.01 \pm 0.032\%$. The tetrazolium test revealed $31.01 \pm 0.072\%$ pollen viability, while fluorochromatic reaction showed 18.28 ± 0.042 (Figure 2 *e*) of the pollen as viable. The plasma membrane of the viable pollen seems to be affected, as the fluorescein leached into the mounting medium (Figure 2 *f*).

The pollen grains germinated in standard Brewbaker and Kwack's medium containing 2.5, 5, 10 and 20% sucrose. No germination was recorded at sucrose concentrations below 2.5% and above 20%. Also, there was no germination when sucrose alone was used as the medium. Maximum germination of $4.55 \pm 0.052\%$ was

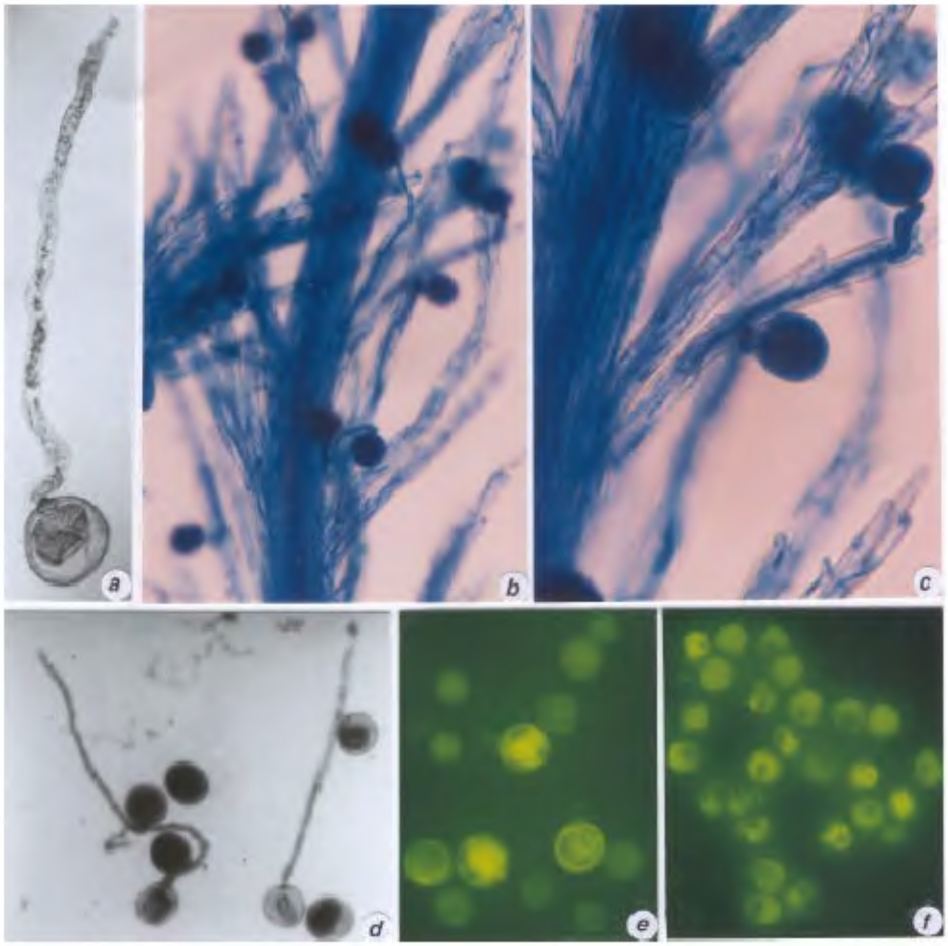

Figure 2. ***a–f***, *Bambusa vulgaris*, pollen viability and germination: ***a***, ***d***, *In vitro* germinated pollen in Brewbaker and Kwack's medium + 10% sucrose; ***b***, ***c***, *In vivo* pollen germination and inhibition of pollen tubes in the stigmatic papillae; ***e***, ***f***, Fluorochromatic reaction test showing viable/non-viable pollen; (***a***, ***c*** × 400; ***b***, ***d***, ***e***, ***f***, × 200).

Table 1. Degree of chromosome associations

No. of PMCs	Quadri-valents	Tri-valents	Bi-valents	Uni-valents	2*n*
1	8	12	6	2	82
3	8	6	7	2	72
1	8	6	7	–	70
9	7	9	8	1	72
9	6	9	8	5	72
6	6	8	10	4	72
1	5	12	8	2	72

Table 2. Chromosomal abnormalities

Nature of irregularity	Mean value (%) irregularities
Non-orientation of chromosome on metaphase plate	96.00 ± 0.05
Laggards	94.01 ± 0.073
Cytomixis	43.01 ± 0.031
Clumping	97.02 ± 0.01

obtained in Brewbaker and Kwack's medium supplemented with 10% sucrose (Figure 2 *a*, *d*).

The percentage of pollen germination on the stigmatic surface following self-pollination was 2.72 ± 0.023 (Figure 2 *b*, *c*). However, all the pollen tubes were inhibited in the stigmatic papillae itself (Figure 2 *c*). There was no evidence of any callosic plug preventing its growth.

The results of acetocarmine test, tetrazolium test as well as fluorochromatic reaction test are in general agreement with earlier reports[5,7].

The various types of chromosome associations (quadrivalents, trivalents, bivalents and univalents) and the high degree of meiotic abnormalities resulting in very low pollen fertility observed, are clearly suggestive of the autopolyploid nature of this taxon. Further, as a consequence of high degree of irregularities in anaphase segregation resulting in production of microspore with varying size and nuclear content were evident, which in turn has resulted in pollen size polymorphism and acute pollen sterility.

Somatic chromosome study of the root-tip cells revealed considerable amount of inconstancy in their diploid constitution. Non-disjunction as well as multipolar

mitosis may account for irregular chromosome distribution in somatic cells. This phenomenon of chromosomal inconstancy (mosaicism) has been reported in a number of vegetatively propagated monocotyledons[16]. Mosaicism is often associated with the occurrence of polyploidy, hybridity and chemical treatment. However, these variations have not resulted in any appreciable phenotypic variation. Some of the gametes behave normally where the complements are balanced, as is often the case in polyploids. Union of these gametes can produce considerable variation in chromosome numbers in the next generation.

Pollen germination and pollen tube growth are prerequisites for fertilization and seed development. An earlier attempt on *in vitro* germination[7] in *B. vulgaris* proved futile. However, the present investigation has shown that at least a very small percentage of pollen grains in this species is viable.

It is pertinent to note that the stigma in *B. vulgaris* is dry and no pollen was found on it under natural conditions[8]. From this, it can be inferred that there are some physical barriers adversely affecting pollination in this species. The inability of the stigma to get exposed or even if exposed, the stigmatic portion being hidden by the staminal filaments and the role of bristle-like hairs on palea preventing the pollen to fall on the stigma, could be reasons for the absence of natural pollination, as reported earlier[6]. The percentage of *in vivo* pollen germination though low, was very significant. In spite of germination, the pollen tube did not find its way into the style, to effect fertilization. This appears to be the result of self-incompatibility. Self-incompatibility can be confirmed only when pollen grains of a different clone are available for effective cross-pollination[17].

Factors responsible for failure of seed set in *B. vulgaris* seem to be manifold. High rate of pollen sterility, absence of natural pollination and inhibition of pollen tubes in the stigmatic papillae act as cumulative factors for lack of seed set in this species.

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Influence of different strains of *Agrobacterium rhizogenes* on induction of hairy roots and artemisinin production in *Artemisia annua*

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Different strains of *Agrobacterium rhizogenes*, viz. A_4, 15834, K_{599}, LBA 9402, 9365 and 9340, were evaluated for induction of transformed hairy roots in *Artemisia annua* using shoot-tip meristem explants. Incorporation of acetosyringone in bacterial culture and co-cultivation medium increased the frequency of hairy root induction in *A. annua*. Different strains of *A. rhizogenes* varied in their virulence for induction of hairy roots. Growth kinetics of transgenic hairy roots induced by different strains indicated a similar pattern of growth, with maximum growth occurring between 14 and 16 days. Transformed hairy root cultures showed significant differences in artemisinin content. A hairy root line induced by strain 9365 was found to contain highest amount of artemisinin (0.23%). Artemisinin content of different hairy root lines was also found to be growth-related.

MALARIA ranks among the most prevalent and severe infectious diseases of the tropics and has been responsible for

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