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Flowering and reproductive biology of two endemic bamboo species of the Western Ghats - *Dendrocalamus stocksii* and *Pseudoxytenanthera ritcheyi*

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Abstract: *Dendrocalamus stocksii*, a common cultivated bamboo species in Konkan area was observed in flowering in Kerala during September 2003 - January 2006. Similarly, *Pseudoxytenanthera ritcheyi*, endemic to the Western Ghats, also flowered in 2006. The offsets of both the species collected previously and planted in Kerala Forest Research Institute campus flowered synchronously. The previous record of flowering of *D. stocksii* dates back to more than a hundred years, while that of *P. ritcheyi* was in 1987-'88. Observations were recorded on flowering behaviour, reproductive biology and seed set in both the species. *P. ritcheyi* showed 98 per cent viability of pollen both in acetocarmine staining and *in vitro* germination tests. Pollen germinated within 15 to 20 min. in the medium containing sucrose, calcium nitrate and boric acid with tube length six times greater than the diameter of the pollen grain. Although pollen grains of *D. stocksii* showed 90 per cent viability in the acetocarmine staining method, *in vivo* germination was absent and *in vitro* germination was very poor (maximum 11%). There was no seed formation in *D. stocksii* and many flowered clumps reverted to vegetative phase. Profuse seed formation was observed in *P. ritcheyi* and flowered clumps died after seed set. Lack of *in vivo* pollen germination could probably explain absence of seed set in *D. stocksii*. Reversion to vegetative phase is a promising trait that can be used for selection of mother clumps for large-scale planting.

Key words: *Dendrocalamus stocksii*, *Pseudoxytenanthera ritcheyi*, reproductive biology, pollen germination, flowering, seed set.

INTRODUCTION

Dendrocalamus stocksii (Munro) M. Kumar, Remesh and Unnikrishnan (=*Pseudoxytenanthera stocksii* (Munro) Naithani, *Oxytenanthera stocksii* Munro) and *Pseudoxytenanthera ritcheyi* (Munro) Naithani. (*Bambusa ritcheyi* Munro, *Oxytenanthera monostigma* Bedd., *Oxytenanthera ritcheyi* (Munro) Blatter and McCann) are medium sized (the former 9 m tall and 2.5 to 4 cm diameter and the latter 3 to 4.5 m tall and 2.5 to 3. 5 cm diameter) bamboo species endemic to the Western Ghats. *D. stocksii* is naturally distributed in the states of Maharashtra,

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Karnataka, Kerala and Goa and grows well in rocky and laterite soils. Since it is widely cultivated in the coastal areas and homesteads of Konkan (Northern Kerala, Karnataka and Maharashtra), it is popularly known as Konkan bamboo. *P. ritcheyi* is distributed in Maharashtra, Karnataka, Kerala and Tamil Nadu. Culms of both the species are used for fencing, making walking sticks, poles, umbrella handles, agricultural implements, baskets and artifacts for cultural and religious ceremonies.

The information on flowering of *D. stocksii* dates back to 1889 from North Kanara (Blatter, 1929). There are no reports on flowering of this species for more than one hundred years. Caryopsis is not reported in this species (Seethalakshmi and Kumar, 1998). First report of flowering of *P. ritcheyi* from Kala Naddi was in 1852, and the recent reports are from Nilambur (1987-'88) and Silent Valley (1995-'96) (Sequiera and Kumar, 1995; Seethalakshmi and Kumar, 1998). No information is available on the reproductive biology and post-flowering behaviour of both the species. In this paper we report information on flowering and reproductive biology of the species.

MATERIALS AND METHODS

Observation on the extent of flowering of *D. stocksii* was made on the clumps distributed in homesteads of Cherkkala, Chattanchal, Poinachi, Chengala, Periyam, Cheruvathur and Kanhangad in Kasaragode District, Kerala State during 2003–2006. To carry out detailed investigation on reproductive biology, rhizomes of the flowered clumps were collected and transplanted in the nursery area at Kerala Forest Research Institute (KFRI) campus, Peechi, in the year 2004. One clump of *D. stocksii* in the KFRI bambusetum, which was raised in 1992 through planting of rhizome collected from Kasaragode District, Kerala, also had flowered and continuous observation was recorded for a period of four years. Rhizome collected from the gregarious flowering area of *P. ritcheyi* flowered during 1987-'88 at Marutha, Nilambur, Kerala planted in KFRI campus in the year 2001 flowered in 2006. When the new sprouts from the rhizome developed into inflorescence, observations on floral morphology, anthesis, pollen viability, germination and stigma receptivity were made.

Flowering behaviour

The details of previous flowering were collected from literature and compiled to see whether determination of flowering cycle is feasible. The number of culms flowered per clump was counted. The time of maturity of female and male phase of the floret, anther emergence and anthesis were recorded. Type of pollination was determined by observing both insects visiting anthers and by placing adhesive tapes near florets during anthesis. The maturity and receptivity of stigma was assessed based on the colour and fluid secretion. Flowers were also fixed in FAA to study the floral structure in detail. Measurements on length of spikelets, florets and anthers were taken.

Post-flowering behaviour

The flowered clumps of *D. stocksii* were observed for three years continuously to note the changes occurring after flowering. Flowering was observed in *P. ritcheyi* only recently and the clumps are under observation.

Pollen viability test

Viability of pollen was tested using acetocarmine stain. The pollen that stained well were considered as viable and the shriveled as non-viable. Pollen at anthesis were selected for the test.

***In vitro* pollen germination**

To test the suitable media for *in vitro* germination of pollen, five different germination media were tested (Table 1). Fresh mature anthers were collected and dusted over clean Petri dishes containing germination medium. While dusting, pollen grains from different anthers were mixed to account for variation. Pollen grains were transferred to cavity slides for observation under high resolution.

One hour after inoculation, the number of pollen grains germinated and the total number of grains per field of view were recorded. Pollens with tubes longer than the diameter of the grains were considered as germinated (Tuinstra and Wedel, 2000). Diameter of the pollen grain and pollen tube length were determined using image analyzer (Leica Q 500MC) in millimeter under 40 x magnifications. Pollen germination percentage was calculated in each medium as proposed by Guangchu (2002). Sixteen fields at random were selected for taking observations.

$$\text{Germination \%} = \frac{\text{Number of pollen grains germinated in 16 fields}}{\text{Total number of pollen grains in 16 fields}} \times 100$$

***In vivo* pollen germination**

To determine *in vivo* pollen germination, stigma was collected a few minutes after anthesis and was kept in 1M NaOH solution for 24 h. It was stained with aniline blue

Table 1. Composition of different media tested for pollen germination

Composition	M1	M2	M3	M4	M5
Sucrose (g)	10	10	10	0	10
Boric acid (g)	0.01	0.01	0	0.01	0
Calcium nitrate (g)	0.03	0	0.03	0.03	0
Distilled water (ml)	100	100	100	100	100

for 1 h, placed over a microscope slide and crushed under a cover slip and observed under the microscope (Ramanayake and Weerawardene, 2003). Stigma collected a few minutes after hand pollination was stained with lactophenol cotton blue to observe pollen grains entangled among the stigmatic hairs.

Observation on seed set

A plastic sheet was spread below the flowered clumps and in the nursery, where the transplanted rhizomes were observed in flower. Observation was made on seed set when the spikelets dried after flowering. The fallen mass was collected regularly and examined for fertile seeds in both the species.

RESULTS

Flowering history and post-flowering behaviour

The details of previous reports of flowering in the two species along with the current flowering are given in Table 2. From the previous flowering records of *D. stocksii*, no definite conclusions on flowering cycle could be drawn. It was interesting to observe that in the first year, one third of the total culms flowered in the clump and during the

Table 2. Records of flowering of *D. stocksii* and *P. ritcheyi*

Species	Flowering years	Location	Reference
D. stocksii	1884, 1888	North Kanara	Blatter, 1929
	1994	Silent Valley	Sequiera and Kumar 1995
	2003-2006	Cherkkala, Chattanchal, Poinachi, Chengala, Periyam, Cheruvathur and Kanhangad	Current report
	2003-2006	KFRI Peechi, Bambusetum	Current report
P. ritcheyi	1870	Satara Ghat	Blatter, 1929
	1884	North Kanara	Blatter, 1929
	1889	North Kanara	Blatter, 1929
	1892	MahabalishwarAhamed Nagar	Blatter, 1929
	1929	Kala Naddi	Blatter, 1929
	1943-44	Agumbe, Ballehalli, Chokkadabyle, Kunda, Sirur, Shimoga Division	Kadambi, 1949
	1945	Aramballi	Kadambi, 1949
	1957-58	Koyna Valley of Satara Districts of Maharashtra	Desai and Subramanian, 1980
	1987	Ambumala, Nilambur	Seethalakshmi and Kumar, 1998
	1995	Silent Valley	Sequiera and Kumar 1995
	2006	Marutha, Vazhikadavu, Nilambur	Current report

second year almost all the culms flowered, while two new shoots turned to vegetative phase. The number of new shoots with vegetative phase increased during third year and by fourth year, the clump resumed vegetative growth (Fig. 1 a, b).

However, two consecutive flowerings of *P. ritcheyi* were observed in 1987-'88 and

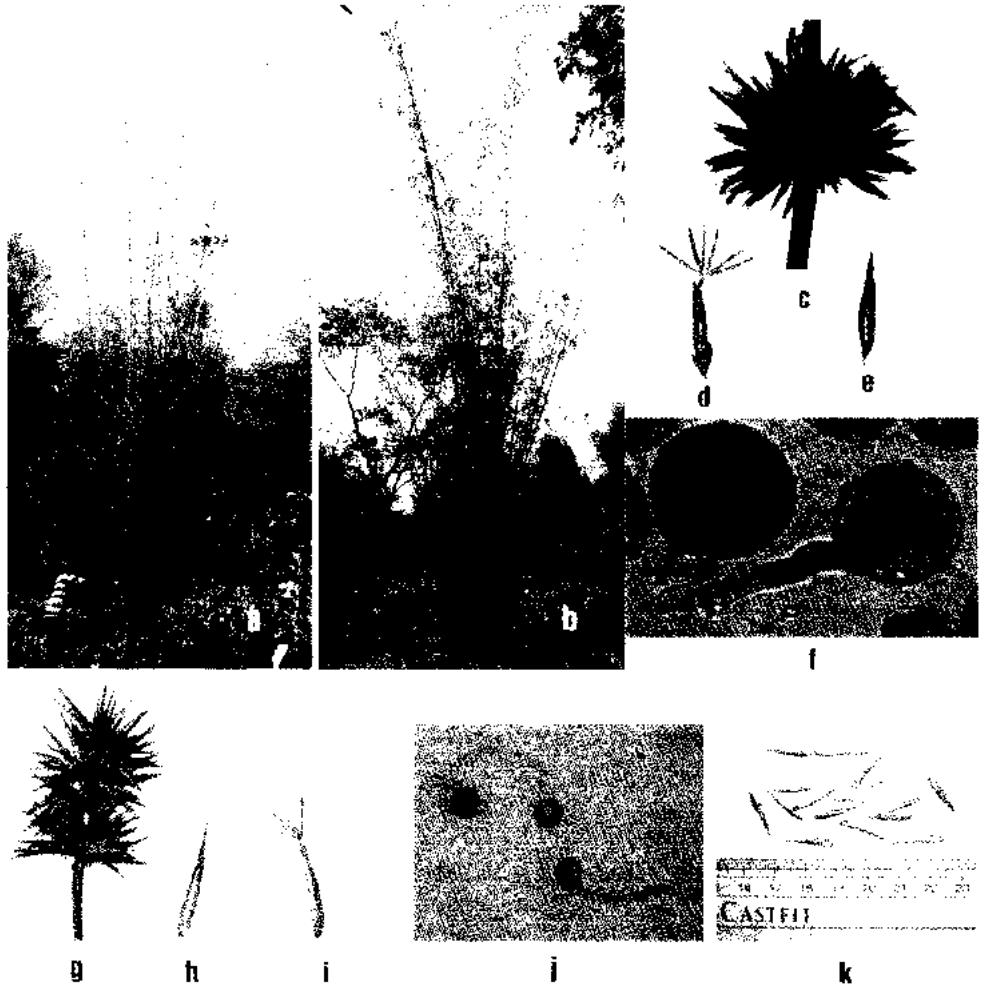

Fig. 1 (a-f) *Dendrocalamus stocksii*

(a) flowered clump
(b) clump reverted to vegetative phase
(c) a portion of flowered branch
(d) spikelet showing anthers
(e) spikelet showing stigma
(f) germinated pollen (40 × magnified)

Fig. 1 (g-k) *Pseudoxytenanthera ritcheyi*

(g) a portion of flowered branch
(h) spikelet showing stigma
(i) spikelet showing anthers
(j) germinated pollen (20 × magnified)
(k) seeds

2006 from the same location at Nilambur indicating that flowering cycle is about 18 years. Flowered clumps of *P. ritcheyi* indicated signs of drying after seed set.

Floral morphology

The details of number and length of spikelets per head, number of florets in female phase and male phase, number and length of anthers and the diameter and tube length of pollen grains are given in Table 3. In both the species, the inflorescence is a panicle with branch-lets bearing semi-globular spicate heads of closely packed spikelets. Each spikelet has 1-2 florets with six stamens and a plumose stigma. The colour of the stigma is purple in *D. stocksii* and white in *P. ritcheyi*. Gynoecium matures 3-4 days before androecium (protogyny) and this prevents self-pollination. Stigma of both species was found emerged out early in the morning around 9 a.m. and remaining receptive during the period of anthesis. The receptivity of stigma was identified by the fluid secretion. Dammer bees (*Trigonia irridipennis*) were found visiting anthers during anthesis; however, they did not visit stigma indicating that bees have no role in pollination.

Anthesis

In *D. stocksii* anthers emerged out from 6 a.m. and dehisced by 11 a.m. Dehiscence was observed only in 15 per cent of anthers while others dried out without shedding pollen grains. Anthers of *P. ritcheyi* emerged at about 9 a.m. and dehisced by 11 a.m. and liberated a large number of pollen. Dammer bees visited and cut open the anthers of *P. ritcheyi* before its full emergence. Pollen grains of both the species are monoporous.

Pollen viability and *in vitro* germination of pollen grains

Ninety per cent of the pollen of *D. stocksii* and 98 per cent of *P. ritcheyi* were found viable when stained with acetocarmine. But both the species behaved differently when pollen grains were germinated *in vitro* in different media (Table 4).

For *P. ritcheyi*, of the five media tested, the medium containing sucrose, boric acid

Table 3. Details of spikelets, female and male phase, anther and pollen grains in *D. stocksii* and *P. ritcheyi*

Particulars	*P. ritcheyi*	*D. stocksii*
Mean number of spikelets per head	25.33 ± 9.5	38.9 ± 4.2
Mean number of florets with exposed stigma per head	2.9 ± 2.07	5.1 ± 1.97
Mean number of florets with emerged out anthers per head	1.9 ± 1.1	3.9 ± 1.37
Mean length of spikelet (cm)	1.81 ± 0.2	1.36 ± 0.09
Mean length of anthers (cm)	0.98 ± 0.04	0.53 ± 0.05
Mean diameter of pollen grains (mm)	0.17 ± 0.02	0.17 ± 0.01
Mean pollen tube length (mm)	0.97 ± 0.12	0.12 ± 0.09

Table 4. Mean germination percentage of *P. ritcheyi* and *D. stocksii* in different medium

Medium	*P. ritcheyi*		*D. stocksii*	
	% germination	Duration (min.)	% germination	Duration (min.)
M1	96.3 ± 3.62	20	11	20
M2	68.68 ± 8.27	20	2	20
M3	95.31 ± 4.34	90	0	-
M4	1.496 ± 1.42	20	0	-
M5	63.95 ± 17.09	20	0	-

and calcium nitrate (M1) gave highest germination percentage (Mean 96.3) followed by medium with sucrose and calcium nitrate (M3). But growth of pollen tubes was very slow when compared to M1 and M2 (20 and 90 min.). The rate of pollen tube elongation was faster in M2 (sucrose and boric acid) but showed only 68 per cent of germination. Germination rate was very poor (1.46) in M4 (no sucrose) and about 63 per cent in M5 (only sucrose).

Generally *in vitro* germination of pollen grains was very poor in *D. stocksii* (maximum 11% in M1 and 2% in M2 and no germination in other media).

Seed set

In *D. stocksii,* seed set was not found in the clump in KFRI bambusetum or in homesteads selected for the study in Kasaragode district, Kerala. Observation of the ground area under flowered clumps after monsoon showers did not show any wildlings indicating complete absence of seed formation in this species. However, good seed production was observed in *P. ritcheyi*; flowered clumps in the nursery at KFRI dried after seed set.

DISCUSSION

D. stocksii was accommodated within the genus *Pseudoxytenanthera* according to previous records (Naithani, 1991). Even though, *D. stocksii* and *P. ritcheyi* showed similarities in habitat and habit, on closer scrutiny, it showed more affinity to the genus *Dendrocalamus* in culm and branching patterns, inflorescence, short apiculate anthers, vestiture of style and feathery stigma and aerial roots in the basal nodes and hence recent nomenclature is *D. stocksii* (Kumar *et al.,* 2004).

From the flowering history, no clue was available about the flowering cycle of *D. stocksii*. Flowering would have occurred between 1888 and 1994 and the flowering cycle of *P. ritcheyi* appears to be 18 years. In 1987, flowering was observed at the same location (Seethalakshmi and Kumar, 1998). There appears to be at least two cohorts of the same species in this locality since some of the patches have not flowered along with the clumps that flowered now.

Pseudospikelets of both the species are bisexual, dichogamous and protogynous as reported for other bamboo species such as *Ochlandra travancorica*, *O. scriptoria*, *Dendrocalamus strictus* (Venkatesh, 1984; Nadgauda *et al.*, 1993; Koshy and Harikumar, 2001). The long duration of 3-4 days between female and male phase ensures that pollination can happen only through pollen grains from other flowers. While observing the possible role of dammer bees visiting the spikelets, they were seen to prefer anthers of *P. ritcheyi* and the anthers were cut open even before they fully emerged out. But the bees were not opening anthers of *D. stocksii*. The bees were not visiting flowers with female phase indicating that there is no role in pollination. The observation of floral structure of both the species reveals that these species are adapted to anemophilous pollination.

Lack of seed production was very obvious in *D. stocksii*. This may be due to reasons like low percentage of anther dehiscence due to quick drying of anthers, lack of deposition of pollen grain in stigmatic hairs and lack of *in vivo* germination of pollen grains. Lack of seed production due to pollen sterility has been reported in other bamboo species such as *Bambusa vulgaris* (Koshy and Pushpangadan, 1997) and *B. balcooa* Roxb. (Banik *et al.*, 1987). Acetocarmine staining showed 90 per cent viability but even 11 per cent germination could be obtained only on addition of boric acid into the germination medium. Boron was added to the medium because it has a regulatory role in pollen germination and pollen tube growth (Wang *et al.*, 2003). It has been reported that no seed set could be obtained when pollen viability is lower than 5 per cent (Wang *et al.*, 2004). The first report on pollen viability and longevity in transgenic forage grasses showed that, under sunny atmospheric conditions, viability of transgenic and non-transgenic pollen reduced to 5 per cent in 30 min, with a complete loss of viability in 90 min. Under cloudy atmospheric conditions, pollen remained viable up to 240 min, with about 5 per cent viability after 150 min (Wang *et al.*, 2004). Whether the climatic conditions at the time of flowering had any negative effect of pollen development in *D. stocksii* is a matter for detailed investigation.

Gregarious flowering and death of flowered clumps after seed set is a common phenomenon in most of the bamboo species. Death of flowered clumps was reported in some of the species like *B. vulgaris* even in the absence of seed production (Koshy and Pushpangadan, 1997). Another report on *B. vulgaris* var. *vittata* shows the reversion to the 'whole green' state (Banik, 1995; Wong, 1995). In *D. stocksii* many of the clumps flowered in Kasaragode and the clump in KFRI bambusetum returned to vegetative phase after flowering. It can be presumed that since there is no seed set and no consumption of reserve food materials for this purpose, such species are able to resume vegetative growth. A detailed investigation on biochemical aspects during flowering and post-flowering stage is required to provide clear evidence regarding post-flowering death/reversion to vegetative phase in bamboos.

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