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FLORAL BIOLOGY AND POLLEN STERILITY IN RELATION TO SEED SET IN *BAMBUSA BALCOOA*

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Bambusa balcooa is one of the priority bamboo species in home gardens of north-east India. Flowering of this bamboo is rarely reported. The species flowered during recent years in Barak Valley of southern Assam, India. The present study reported the floral biology and pollen sterility of *B. balcooa* in relation to seed set. The study revealed that insufficient separation of palea and lemma, non-exposure of essential whorls, low pollen viability and reduced stigma receptivity prevented seed set. Since this species is monocarpic, absence of seeding questions continuity of the species.

Keywords: Bamboo flowering, seeding, pollen viability, Barak Valley

INTRODUCTION

Unusual flowering conduct and long, irregular inter-seeding periods raise concern about bamboo regeneration through seeds. Usually, bamboos flower and seed once at the end of their life time, but some species do not seed even after flowering (Koshy & Pushpangadan 1997, Koshy & Jee 2001). *Bambusa balcooa* (known in India as sil baruah) is an economically important species and has high potential for carbon sequestration and climate change mitigation (Nath et al. 2009, Wang et al. 2013), but it does not set seeds. *Bambusa balcooa* is one of the priority bamboo species identified by the International Plant Genetic Resources Institute and International Network for Bamboo and Rattan (Rao et al. 1998), and is widely used in scaffolding, construction of bridges, agricultural implements and furniture.

Flowering of *B. balcooa* is rarely reported. The available flowering records are listed in Table 1. The recent (2016) sporadic flowering of *B. balcooa* in Cachar (Rosekandy tea estate 24° 41' N, 92° 40' E, Irongmara 24° 41' N, 92° 44' E) and Karimganj (Purba Harinagar 24° 32' N, 92° 26' E) districts of Barak Valley, Assam was monitored scientifically. Daily observations continued in ex-situ planted offsets at Assam University Nursery and Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Kerala. This paper discusses the possible reasons for sterility in *B. balcooa* due to spikelet malfunctions.

Table 1 Records on incidence of flowering for *Bambusa balcooa* in India and Bangladesh

Country	Locality	Year
India	Purnea, Bihar	1849
	Gorakpur, Uttar Pradesh	1881
	Eastern Uttar Pradesh	1986
	Goalpara District, Assam	1876
	Kamrup District, Assam	1889
	Khoraghat Village, Dhubri District, Assam	1998
	Rosekandy, Cachar District, Assam	2014
	Irongmara,Cachar District, Assam	2016
	Purba Harinagar, Karimganj District, Assam	2016
Bangladesh	Sporadic	1977–1985
	Gregarious	1983–1985

Floral structures of bamboos are either of the dendrocalamus-type (i.e. closed) or of the bambusa-type (open) (Koshy et al. 2001). In the dendrocalamus-type, florets constituting each spikelet are dichogamous (Lloyd & Webb 1986) and protogynous in nature, where the palea and lemma do not open to expose the essential stages (i.e. gynoecium and androecium), but instead remain as apically tapering tubes, shut with overlapping glumes (Koshy et al. 2001). Here, style and stamens are exposed by an internal push resulted from their own spontaneous and rapid growth. In the bambusa-type, florets constituting spikelets are homogamous, with both the stigma (female) and anthers (male) exposing simultaneously through the widely separated palea and lemma.

After flowering, culms of *B. balcooa* die without setting seeds (Seethalakshmi & Mukteshkumar 1998, Barooah & Borthakur 2003). *Bambusa vulgaris* also does not seed upon flowering and the reasons for this were investigated by Koshy and Jee (2001). The absence of seeding in *B. balcooa* was previously reported by Banik and Alam (1987).

MATERIALS AND METHODS

Field study

To record flowering incidences in *B. balcooa*, the field area, Barak Valley (Cachar, Karimganj and Hailakandi districts of Assam), was extensively surveyed from 2010 onwards. The first and second authors came across flowering of *B. balcooa* at Purba Harinagar, Karimganj District of Assam on 15th March 2016. Here a single clump was spotted in flower, near a rice field. Specimens were preserved and healthy, live offsets collected (Figure 1a) were transported and planted at Assam University Garden and JNTBGRI for close monitoring. The potted flowering offsets were hardened (voucher no. 80582) and kept in isolation for further study. When the flowering species was collected earlier (on 15th March 2014) from Rosekandy and brought to JNTBGRI (voucher no. 64546), it was mistaken as a *Dendrocalamus* species due to observance of apparent dichogamy and insufficient representation of vegetative structures due to felling. In the present instance also, spikelets in the flowering clump were observed to be of the dendrocalamus-type, thick, lanceolate and laterally compressed (Figure 1b). No female stages were observed, but presence of a few dried anthers, indicated past male stages.

Study on ex-situ planted offsets

To study the floral morphology, flowers were observed from early morning (6 a.m.). Some flowers were preserved in 5% formalin and used for dissection in the laboratory. Spikelets and their dissections were photodocumented. Floral parts were separated and examined under compound microscope and their characteristics recorded.

Pollen fertility was tested using 2% acetocarmine stain, following Shivanna and Rangaswamy (1992). Pollen viability was assessed using tetrazolium test. 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 a humidity chamber at room temperature in the dark for 30 min. The pollen were observed under microscope and stained grains were regarded as viable. Stigma of *B. balcooa* was selfed and crossed manually, using pollen from available *Bambusa* spp. in flowering to test pollen viability and stigma receptivity.

RESULTS

Hardened offsets continued spikelet production but seldom exposed the gynoecium and androecium. From the second week of April till that of May (2016), stigma and stamen exposure were only 5%, that too in separate florets, as if were dichogamous. If emergence occurtred from any of the 5–8 florets in a spikelet, it was mostly from the apical ones (Figure 1d). The present study corroborates findings of John et al. (1994) and John and Nadagauda (2002).

When dissected, florets with mature exposed stigma showed equally mature anthers, held inside (Figure 1f). Florets with exposed anthers also had mature stigma either in partially exposed or in unexposed condition. Thus, it became clear that the florets are homogamous, with the style and stamens remaining unexposed or underexposed, and decaying within (Figure 1e), where normal exposures are a rarity. Longitudinal sections also ruled out the protogynous and dichogamous nature of florets. The closely clasped hairy palea and lemma had failed to separate, widen and expose the female and male reproductive structures properly (Figures 1c and e).

We collected pollen from the rarely emerged anthers of *B. balcooa* offset and selfed it on its own exposed stigmas and also cross-pollinated on stigmas of *Bambusa tulda*, *B. mizorameana*, *B. bambos* and *B. cacherensis*, which were simultaneously available in flower at the Bambusetum of JNTBGRI. None of them seeded, indicating non-viability of pollen. Artificial selfing and

Figure 1 *Bambusa balcooa*—(a) excavated offset, (b) ripe, unopened spikelet, (c) partially exposed stigma, (d) rare exposure of stamens, (e) longitudinal section of an unopened spikelet, (f) dissected, opened spikelet showing homogamy and (g) fungal infection resulted from anther decay inside

crossing of stigma of *B. balcooa*, using pollen from these *Bambusa* spp. also did not result in seeding, indicating non-receptivity of stigma as well. Therefore, it was clear that physical and physiological barriers prevented seed set in this bamboo species.

Fertility test, using acetocarmine, showed 10–15% pollen was fertile, while viability test, using tetrazolium, proved 5–8% pollen was viable. In high seed setting bamboos such as *Schizostachyum dullooa* and *Ochlandra travancorica*, pollen viability rates were 95–98% and 90–95% respectively (Gopakumar 2015).

Very few stigmas and anthers were exposed during the monsoon rains which started on 19th May 2016 (lasted up to 30th September 2016). During the rainy season, even the live spikelets in the flowering offset had fungal infection (Figure 1g). Usually only dead or functionless floral parts of bamboo get fungal infection. Here, it is clear that, unexposed style and stamens, trapped inside glumes, had invited fungal growth. More fungal growth was observed in the continuous rains of July which further retarded spikelet developments. Health of the flowering offset, and thus of its branches, deteriorated day by day. By the last week of August, its 5-month long flowering in captivity ended.

DISCUSSION

In *B. balcooa* studies of floral biology and pollen sterility in relation to seeding is rare. *Bambusa balcooa* does not set seeds because of the long, irregular flowering cycle, pollen sterility, failure of floral glumes to function normally, improper development and unhealthiness of female and male reproductive parts. Similarly, *B. vulgaris* also does not seed due to inherent unhealthy nature of stigma, peculiar opening and closing of florets, presence of bristle-like hairs (ciliate) on palea, high pollen sterility, absence of natural pollination and inhibition of pollen tubes in stigmatic papillae (Koshy & Pushpangadan 1997, Koshy & Jee 2001). In *B. balcooa,* the inherent weakness of style and staminal filaments to expose stigma and anthers respectively, blocked seed set. Sporadic flowering can hinder seed set, and this was observed in *Chusquea talamancensis* (Widmer 1998). Whereas, in gregarious *Dendrocalamus membranaceus* and *D. sinicus* self-compatibility, thus seeding, is reported, with mixed-mating systems and predominant outcrossing (Chen 2017).

Death of bamboo plants after flowering is not inevitable, and reversions to vegetative phase may occur in some (Jijeesh et al. 2009). During off-year bamboo flowering events, mating opportunities and pollen availability are restricted (Mizuki et al. 2014). This was also observed in *B. balcooa*, in addition to structural and functional floral abnormalities. Only 15% anthers of *Dendrocalamus stocksii* (also a non-seeding prioritised solid bamboo like *B. balcooa*) dehisce and release pollen, but with very low viability (Beena 2012). Dichogamy and protogyny are reasons described for absence of seed set in *D. stocksii* (Viswanath et al. 2012). However, this might not be the sole reason considering seed set in the dichogamous and protogynous *Dendrocalamus strictus* (Nadgauda et al. 1992). In contrast with *B. vulgaris* and *D. stoksii, B. balcooa* is proved monocarpic due to death of clumps after flowering. Flowering clumps and offsets of 2014 and 2016 have died down after completion of the process. Absence of seed set terminates the life of *B. balcooa.* This necessitates planning of adequate and effective conservation strategies for *B. balcooa.*

CONCLUSIONS

Insufficient or non-exposure of stigma and stamens, improper separation of ciliated lemma and palea, reduced pollen fertility and viability, and poor stigma receptivity were reasons for the failure of seed set in *B. balcooa*. The available information raises concern about conservation of this priority species due to death (monocarpy), without seeding.

REFERENCES

Banik RL & Alam MK. 1987. A note on the flowering of *Bambusa balcooa* Roxb. *Bano Bigyan Patrika* 16: 25–29.

Barooah C & Borthakur SK. 2003. *Diversity and Distribution of Bamboos in Assam.* Bishen Singh and Mahendra Pal Singh, Dehradun.

Beena VB. 2012. Reproductive biology and biochemical changes associated with flowering of *Dendrocalamus stocksii* and *Ochlandra travancorica.* PhD thesis, Cochin University of Science and Technology, Cochin.

Chen LN, Cui YZ, Wong KM, Li DZ & Yang HQ. 2017. Breeding system and pollination of two closely related bamboo species. *AoB PL,*plx021.

Gopakumar B. 2015. Studies on the ecology and conservation of *Ochlandra travancorica* and *Ochlandra wightii*, major two reed bamboos of Kerala, endemic to South India. PhD thesis, University of Mumbai, Mumbai.

Jijeesh CM, Seethalakshmi KK, Beena VB & Raveendran VP. 2009. Recent flowering of an endemic bamboo—*Pseudoxytenanthera monadelpha* (Thw.) Soderstrom and Ellis in Munnar, India. *Phytomorphology* 59: 35–39.

John CK, Nadagauda RS & Mascarenhas AF. 1994. Reproductive biology: an aid in the classification of bamboos. *Current Science* 67: 685–687.

John CK & Nadagauda RS. 2002. Flower structure and function in the classification of bamboos. *Current Science* 82: 1309–1310.

Koshy KC & Jee G. 2001. Studies on the absence of seed set in *Bambusa vulgaris. Current Science* 81: 375–378.

Koshy KC & Pushpangadan P. 1997. *Bambusa vulgaris* blooms, a leap towards extinction? *Current Science* 72: 622–624.

Koshy KC, Harikumar D & Narendran TC. 2001. Insect visits to some bamboos of the Western Ghats, India. *Current Science* 81: 833–838.

Lloyd DG & Webb CJ. 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms. I. Dichogamy. *New Zealand Journal of Botany* 24: 135–162.

Mizuki I, Sato A, Matsuo A, Suyama Y, Suzuki JI & Makita A. 2014. Clonal structure, seed set and self-pollination rate in mass flowering bamboo species during off-year flowering events. *PLoS* 9: e105051.https://doi.org/10.1371/journal.pone.0105051.

Nadgauda RS, John CK & Mascarenhas AF. 1992. Floral biology and breeding behavior in the bamboo *Dendrocalamus strictus* Nees. *Tree Physiology* 13: 401–408.

Nath AJ, Das G & Das AK. 2009. Above ground standing biomass and carbon storage in village bamboos of north east India. *Biomass and Bioenergy* 33: 1188–1196.

Rao AN, Rao VR & Williams JT. 1998. *Priority Species of Bamboo and Rattan*. International Plant Genetic Resources Institute–Regional Office for Asia, the Pacific and Oceania, Serdang.

Seethalakshmi KK & Mukteshkumar MS. 1998. *Bamboos of India, A Compendium*. Kerala Forest Research Institute, Peechi and International Network for Bamboos and Rattan, Beijing.

Shivanna KS & Rangaswamy NS. 1992. *Pollen Biology: A laboratory Manual*. Springer, Berlin–Heidelberg.

Viswanath S, Geetha J, Somashekar PV, Ajay DR, Soumya C & Joshi SC. 2012. *Dendrocalamus stocksii (Munro): A Potential Multipurpose Bamboo Species for Peninsular India*. IWST Technical Bulletin No. 10. Institute of Wood Science and Technology, Bangalore.

Wang B, Wej WJ, Liu CJ, Niu X, & Man RZ. 2013. Biomass and carbon stock in moso bamboo forests in subtropical China, characteristics and implications, *Journal of Tropical Forest Science* 25: 137–148.

Widmer Y. 1998. Flowering phenology of *Chusquea* bamboos with special reference to *Chusquea talamancensis* in Costa Rica. *The Journal of American Bamboo Society* 12: 1–20.