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A detailed review on bamboo seed: Emphasizing seed set, viability enhancement and storage method

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Abstract
Bamboo is considered a very important plant species for its increasing demand in various industries and market. Seed availability in bamboo is uncertain due its irregular flowering habit and less viability. A review is discussed on bamboo seed set problems, different germination enhancement treatments and seed storage protocol. Seed treatments like infusion, osmopriming and fortification, pelleting, hardening etc are used to enhance germination in bamboo seed. Different storage materials are used at various temperature levels for storing bamboo seed.

Keywords: Bamboo seed set, viability enhancement, storage

Introduction
Bamboo plant, termed as "Green Gold", "Poor Man's Timber", "Bamboo, Friend of The People", "Cradle to Coffin Timber" for its diversified uses in various field. It is used for various purposes ranging from construction work, edible shoot, charcoal preparation, cottage industries and many more. Besides these it helps in maintaining and improving soil physical, chemical and biological properties due to high biomass accumulation (Shanmughavel *et al*., 2000) [27]. Bamboo plants are both cultivated and grow wild in the forest areas. Total bamboo bearing area is estimated to be 15.69 million ha in India. Most of the bamboo population is concentrated in deciduous and semi evergreen regions of North East and the tropical moist deciduous forests of North and South India. North-eastern hilly states of India are home for nearly 90 species of bamboo, out of which 41 species are endemic to this region (Tewari *et al*, 2019) [32]. The phenomenon of flowering continues subsequent generations in angiosperm. Some of Poaceae family members have suicidal habit, where the parent plants die after flowering. Bamboo being a member of the sub-family Bambusoideae of poaceae exhibits similar characteristics (Singha *et al*., 2003) [30]. Due to irregular and long flower cycle research on bamboo seed is very limited in the literature. Bamboo produces seeds either sporadically or greagariously. It produces one seeded fruit known as caryopsis covered with number of persistent glumes (Gamble, 1896, Gould, 1968) [10, 11]. Mass multiplication of bamboo is needed for its high market demand. Both vegetative propagation and seed multiplication system is used for regeneration in bamboo. Seed production is restricted to only some bamboo species. In some species due to irregular flowering habit there is scarcity in seed availability. Even if seeds are available bamboo seed viability is very low. Factors like uneven germination, low desiccation tolerance reduces seed longevity and hence viability. These are the main problems of bamboo multiplication through true seed.

Problems that inhibit the seed setting in some bamboo species
There are two types of floral structures in bamboo. Dendrocalamus or closed type posses dichogamous and protogynous florets. In this type lemma and palea do not open to expose the essential stages but remain as apically tapering tubes shut with overlapping glumes. Bambusa or open type floral structure constituting homozygous spikelets exposes both anther and stigma simultaneously through widely separated lemma and palea (Koshy *et al*, 2001) [13]. Some of the species has not flowered till date and some are monorcarpic which dies after flowering and seed setting. Reports related to cause of less seed setting in different species of bamboo is very rare. Some of the findings on bamboo seed set problems are reviewed and discussed. Das *et al*., 2017 [9] discussed the possible reason for sterility in *Bambusa balcooa* due to spikelet malfunction. Ex-situ planted flowering offsets were taken up for the study. The spikelet in the flowering clump was observed to be dendrocalamus type which were thick, lanceolate and

laterally compressed. A few dried anthers were present indicating past male stages but there was no presence of female stage in the flowering clump. There was only 5% exposure of androecium and gynoecium in the apical part that too in separate florets indicating dichogamous nature of the flower. Flowers were preserved in 5% formalin are dissected and observed under the microscope for recording their characteristics. Mature florets were dissected and it was observed that *B. balcooa* florets are homozygous. Both style and stamen of *B. balcooa* flowers remain unexposed or underexposed and was decayed inside. Closely clasped Hairy lemma and palea had failed to separate, widen and expose the reproductive structures properly in *B. balcooa* species. Pollen fertility testing was conducted using 2% acetocarmine stain (Shivanna and Rangaswami, 1992) [28] and only 10-15% pollen was found fertile. Pollen viability was recorded to be 5-8% when tested with tetrazolium. To test pollen viability, pollen from *B. balcooa* was collected and selfed on its own exposed stigma and also cross pollinated with other bamboo species viz., *B. tulda, B. mizorameana, B. bamboos* and *B. cacherensis*. It was observed that none of the floret produced seed indicating non-viability of pollen. Moreover pollen from other *Bambusa* spp. (*B. tulda, B. mizorameana, B. bamboos* and *B. cacherensis*) were collected and crossed with *B. balcooa* but no seed setting was found and hence resulting non receptivity of stigma in this species. In this experiment fungal growth was also observed in unexposed style and stamen trapped inside glumes. As *B. balcooa* is a monocarpic plant absence of seed setting terminates life of this bamboo species. This depicts the importance of planning adequate and effective conservation strategies for *B. balcooa*. Bhattacharya *et al*., 2006 [5] conducted an experiment on *Bambusa tulda* to describe its morphological characters (vegetative and reproductive) as well as molecular markers in order to enable species identification at various stages of life cycle. For this study five different fertile florets from each inflorescence were selected and measurements were taken. There was no record of *B. tulda* seed production in the present study. The author revealed that *Bambusa tulda* seed doesn't set may be either due to protandry or due to the short height of the pistil coupled with tight overlapping of lemma and palea, preventing the pistil from coming out, thereby reducing the chances of cross-pollination. In an experiment on *Bambusa cacharensis* flowering was not followed by production of viable seed. Pollen grain of *B. cacharensis* was studied for their sterility and viability and 70% pollen was found fertile and viable. Singha *et al*. 2003 [30], revealed that due to short gynoecium and unsticky nature of the stigma resulted in failure of fertilization and production of seed. This natural phenomenon has contributed to a reduction of the bamboo resource. Factors responsible for failure of seed set in *Bambusa vulgaris* through studies on pollen sterility, viability, *in vitro* and in vivo germination, in addition to cytology was carried out by Koshy and Jee, 2001 [13]. Pollen morphology was studied and pollen size classes were recorded by using Walker and Doyle method. Pollen sterility was determined by using 1,1 mixture of glycerin and 2% acetocarmine. The degree of pollen sterility in B. vulgaris was found 71.01±0.032%. Pollen viability was measured using tetrazolium and calculated as 31.01±0.072%. Moreover fluorochromatic reaction revealed 18.28±0.042% pollen viability. Maximum *in vitro* pollen germination (4.55±0.052%) was obtained in Brewbaker and Kwak's medium supplemented with 10% sucrose. In vivo germination study was carried out on field grown plants by controlled self pollination. In this process pollen germination and pollen tube growth was observed in self pollinated *B. vulgaris* floret. The percentage of pollen germination in stigmatic surface was calculated as 2.72±0.023 and all the pollen tubes are inhibited in stigmatic papillae. The author also opined that high degree of meiotic abnormalities resulted in low pollen fertility and irregularities in anaphase segregation led to pollen size polymorphism and acute pollen sterility. Under natural condition stigma of *B. vulgaris* was dry and no pollen was found in the stigma indicating some physical barrier adversely affecting pollination in the bamboo species. Moreover non exposure of stigmatic surface due to staminal filament and prevention of pollen to reach stigma by bristle like hair on palea are some other reasons of no seed set in *B. vulgaris*. In this study it was concluded that factors responsible for failure of seed set in *Bambusa vulgaris* were high rate of pollen sterility, absence of natural pollination and inhibition of pollen tubes in the stigmatic papillae.

Viability enhancement treatment

Bamboo seeds are viable for very less duration. Literature on cause of less viability is very limited in seed of bamboo. Ageing in bamboo seed leads to two major changes in the metabolism. One change is hormonal imbalance causes rapid loss in seed germinability which can be recovered using exogenous PGRs upto certain age of the seed. The other change noticed was slow and irreversible deterioration of membrane integrity which leads to permanent loss of bamboo seed viability (Richa *et al*., 2010) [24]. Seed health is considered limitation for sexual propagation of bamboo plant through its true seed. External or internal born microbs reduces bamboo seed germination and hence reducing the seed quality and seedling vigor. In India and Thailand a total of 65 fungi belonging to 37 genera and two bacteria have been reported on stored seeds of different bamboos species (Renganayaki *et al*., 2018) [22]. Therefore proper seed treatment prior to nursery raising is required to reduce the problem of pathogenicity and its further effect on bamboo seedling emergence and growth. A thorough review is done on enhancement treatments developed by different researchers.

Seed pathology studies in *Bambusa bambos* was done by Renganayaki *et al*., 2018 [22]. Bamboo seed collected from ground are often infected with soil borne pathogens which invades during seed germination and causes secondary infection. These pathogens multiply during seed storage and reduces its storage life and viability. Seed health test was conducted in *Bambusa bambos* and predominant species observed were *Alterneria* spp., *Fusarium spp*., *Aspergilus flavus* and *Aspergilus niger* (39% infection and less germination of 62%). Seeds were treated with different fungicides (Bavistin, Ridomil and Captan) both as wet and dry treatments and surface sterilization chemicals (Thiourea and Mercury Chloride). This study suggested soaking *B. bambos* seeds in 0.5% Bavistin solution for 1h, dried back to original moisture content and dry dressing with 4 g of Captan kg^{-1} of seeds which effectively control the surface borne pathogens. Application of this treatment reduced pathogen infection to 5% and increased germination percentage to 78%. Richa *et al*., (2018) [23] studied membrane integrity deterioration with seed viability or physiology during storage of *Dendrocalamus hamiltonii* seed under both natural ageing (room temperature) and controlled ageing (desiccators at 4

^{0}C) and to understand the effect of invigoration treatments on its viability. *Dendrocalamus hamiltonii* seeds were exposed to both pre sowing (infusion, osmopriming and fortification) and post storage treatment (pelleting and hardening). For infusion treatment GA_3, IAA, IBA at different concentration of 10, 20, 50 ppm was used. Osmopriming was conducted using KCL, KNO_3 and PEG-6000 at different rates of 2%, 5% and 10%. Seed fortification was done using Ascorbic acid (2%, 5% and 10%), GA_3 (10, 20, 50 ppm) and KH_2PO_4 (2%, 5% and 10%). Among these treatments best pre sowing treatment for *Dendrocalamus hamiltonii* seed was found to be GA_3 @50 ppm that showed 26% germination after 18 months of ageing. *Dendrocalamus hamiltonii* seed palleting was done by using Calcium Oxychloride, Calcium Carbonate, Turmeric powder @2, 5, 10% respectively. Its seed hardening was done by using GA_3, IAA, Albizzia leaf powder, Clay and Calcium oxychloride applied at 2%, 5% and 10% respectively. Turmeric concentration at the rate of 10% was found best pre storage treatment having germination of 24.9% after 18 months of storage. Both pre sowing and post storage treatments are statistically significant in improving viability in *Dendrocalamus hamiltonii* seed. Seed invigouration treatment can improve seed shelf life to a great extent. Mid –storage treatment and its effect on *Bambusa bambos* seeds was experimented by Renganayaki *et al*., 2017 [21]. Water, chemical and vitamin solutions were used as treatments in *Bambusa bambos* seeds. Para Amino Benzoic Acid, Para Hydro Benzoic Acid, KH_2PO_4, 500ppm α – tocopherol, 600ppm α – tocopherol are used as treatment at various concentrations applied for different time interval. The experiment revealed that water soaking for 4h and drying improved germination (91%) and vigour (1738) immediately after treatment when compared to chemical and vitamin solutions. From the observations, water soaking for 4h and drying can be recommended as no cost mid storage seed treatment for better performance of *B. bambos* seeds. In another experiment on effect of pre-sowing invigouration treatments on performance of ageing *Dendrocalamus strictus* seeds treatments used were Infusion, Fortification and Osmopriming with various bioactive chemicals (Singh *et al*., 2015). Infusion was done with GA_3, IAA, IBA @ 10, 20, 50 ppm each and PEG-6000 with 2%, 5%, 10% concentration. Similarly fortification was done with Ascorbic acid and Potassium dihydrogen phosphate (2%, 5% and 10% conc.) and GA_3. Osmopriming was done with KCl, KNO_3 and PEG-6000. It was found that GA_3 (50 ppm) was most effective in maintaining germination percentage and vigour index of both fortified and infused seeds. Among all the osmopriming treatment, KCl (10%) resulted in best germination percentage (83.1%) and vigour index. It is concluded that seed invigouration treatments offer promising results in order to regain seed viability before sowing. *In vitro* propagation protocol in *D. membranaceus* using mature seed was developed in an observation (Brar *et al*., 2012) [7]. Different germination experiments were carried out to study the effect of sterilants, light conditions, plant growth regulator including role of gibberellic acid (GA_3) and temperature in overcoming germination barrier in aged bamboo seed. Fungal and bacterial contamination is very much common in bamboo seed. Seed requires longer duration of treatment with antimicrobial chemical (Thakur and Sood, 2006) [33]. Study revealed that $HgCl_2$ (0.1%) along with bleach (15%) for 10 minutes was found effective in raising 77.8±9.6% aseptic cultures establishment. GA_3 applied at the rate of 50 ppm showed maximum germination of 73.3±5.7%. Along with this shoot length and number of sprouts were also found increasing after two weeks. GA_3 activates the metabolism to initiate sprouting in seed by producing and secreting hydrolytic enzymes from aleuron layer. Bamboo seeds are considered as negatively photoblastic (Banik, 1996) [3]. Maximum germination was obtained in dark condition, high temperature around 30 ^{0}C (72±9.6% germination) and overnight seed soaking in GA_3 @ 50 ppm (73.3±5.7% germination). The study also revealed that seeds were found non-viable after one year when tested with Tetrazolium chloride test. A study was conducted to improve viability of ageing *Dendrocalamus strictus* seed by using plant growth regulator and study the role of enzymes promoting seed germination in relation to exogenous PGRs (Richa *et al*., 2010) [24]. Plant growth regulator used for the experiment were GA_3 (40 ppm), IBA (20 ppm), resorcinol (20 ppm) and 1,2,4-acid (20 ppm). Initially all the treatment combination increased α and β amylase activities in seed but after six months of ageing only IBA treatment was found to be effective in germinability and enzyme activity.

Storage methods to retain seed viability

Seed longevity in bamboos varies species to species. Increased aging of seeds and natural conditions generally hampers the viability of seeds. Bamboo seeds have very short viability and therefore it can be useful to propagate for only a short period of time. Endogenous levels of auxin and abscisic acid is found to be one of the major factor for loss of viability in stored bamboo species (Richa *et al*., 2006) [25]. Viability of bamboo seeds depends upon different factors such as storage condition, time, moisture content, temperatures, pathogen attack during the storage period and so on. Researchers have developed lots of storage conditions and procedures for storing bamboo seeds for longer period without losing viability and vigor. Different seed storage strategies have listed out from collected literature. Those are as followed-

Table 1.1: Different storage methods for bamboo seeds

Species	Storage temp. (°C)	Storage moisture (%)	Materials	Viable for (months)	Viability %	References
Bambusa arundinacea	-3 °C – 0 °C	-	over anhydrous calcium chloride	413 days	-	Somen and Seethalakshmi, 1989 [31]
	-70 °C	-	sealed polythene bags	12	65	Midya, 1994 [16]
	low temp	1.9	-	18	6	Warrier *et al*., 2004 [38]
	8 °C -12 °C	10	-	12	-	Mahadaven *et al*., 2003
Bambusa bamboos	at low temp	10 to 11	Using desiccant	More than 1 year	-	Somen and Seethalakshmi, 1989 [31]
Bambusa tulda	15 °C	6.6	sealed polythene bags	12	70	Thapliyal *et al*.,1991 [35]
	5 °C, 15 °C	10.2 and 6.6	-	240 days	20	Thirtha *et al*., 2013 [36]
Chimonocalamus	4 °C	-	-	-	-	Yang *et al*., 2013 [39]

pallens						
Dendrocalamus. asper	-20 ºC	4.34	-	12	69.33	Thapliyal and Kainthola (2013) [34]
Dendrocalamus brandisii	4 °C	8	sealed double polythene bags(gauge 0.05mm) placed in airtight plastic containers	30	-	Lakshmi *et al*., 2014 [14]
	2°-4 °C	-	-	18	-	Boonarutee and Somboon, 1990 [6]
Dendrocalamus membranaceus	low	less	-	45 years	-	Rawat and Thapliyal, 2003 [19, 20]
Dendrocalamus strictus	3º-5 ºC	8	over blue silica gel in dessicator or anhydrous calcium chloride in dessicator or in sealed glass bottle.	34	-	Gupta and Sood, 1978 [12]
	0º to 5 ºC	8.4	-	9	-	Ravikumar *et al*., 1998 [18]
	5º and -5 ºC	2.8 - 8.9	airtight containers	3years	-	Rawat and Thapliyal, 2003 [19, 20]
Meloccana baccifera	-	-	dry sand	60	-	Banik, 1994
Oxytenathera abyssinica	-	-	glass bottles	2 years	--	Ayana *et al*., 2012 [1]
Thamnocalamus spathiflorus	25 °C	8 to 9	-	4 years	93.3	Bag and Palni, 2013 [2]
Thyrsostachys oliveri	-4 ºC	-	-	18	-	Seethalakshmi and Muktesh Kumar, 1998 [26]
Thyrsostachys siamensis	-5 °C and 2°-4°	6 to 10	-	-	-	Ramyarangsi, 1988 [17]

Note: - indicates particular parameter was not considered for the respective study

Conclusion

Increase in demand for bamboo industry throughout the world increases the demand for quality seed production and its timely supply to the bamboo growers. It is very necessary to promote bamboo cultivation through appropriate methods. Bamboo species vary in flowering habit, seed production and germination. There is continuous effort of bamboo researchers in finding methods to increase the viability and developing standard seed storage method for bamboo. Its long flowering cycle, no seed set and less viability still remain mystery in some species. More research is needed in bamboo seed production for mass multiplication and bamboo variations so that the full potential of bamboo plant could be utilized.

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