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Optimum conditions for testing germination of bamboo seeds

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Editorial note:
Unfortunately the author of this article passed away before he could use the recommendations by the reviewers for his revised article. To pay respect to this scientist and in view of the importance of the information in this article, it is published with editing only. On the next page you will find an Obituary, followed by the article.

JULES JANSSEN

Dr. Man Mohan Singh Rawat

Obituary

Dr. Man Mohan Singh Rawat
(31 December 1956–1 March 2004)

It is with a deep sense of grief to learn of the sudden passing away of Dr. M. M. S. Rawat after coronary artery heart by-pass surgery at the young age of 47 years. This came as a great shock. Born in 1956 at Dehra Dun (Uttaranchal), India, he joined the Forest Research Institute, Dehra Dun in 1977 as Research Assistant after his MSc in botany in 1976 from H. N. B. Garhwal University. He was awarded the DPh degree in Forestry Seeds in 2001 from FRI Deemed University, Dehra Dun. In 1996 he was appointed as Research Officer at Forestry Seeds Laboratory, Forest Research Institute, Dehra Dun. He had very good and vast research experience in forestry seeds research. He was appointed as Scientist-B and joined the Arid Forest Research Institute, Jodhpur (Rajasthan) on 13 June 2002. He has about 30 research papers published in various national and international repute journals to his name. He was working on the seeds quality and their improvement for arid zone forestry in India from June 2002 and felt ill in the last week of November 2003 after diagnosis of coronary artery heart disease. In December 2003 he went to New Delhi for by-pass surgery. While recovering his health at Dehra Dun he passed away on 1 March 2004 after sudden heart failure.

Dr. M. M. S. Rawat was a person of quiet disposition, hard working, amiable and helpful for staff, colleagues and friends. His wife, Mrs. Neelam Rawat, a teacher at St. Thomas College, Dehra Dun, was a source of great strength to him.

His wife Mrs. Neelam Rawat and only daughter, Miss Vanushri Rawat, who is doing her study for a bachelor degree in engineering at Dehra Dun, survive him. Dr. M. M. S. Rawat will be a great loss to the science community as a whole and especially in forestry seeds in India. He will be also greatly missed by his relatives, friends and colleagues.

May his soul rest in peace.

Abstract—Studies were conducted on seeds of three bamboo species, *viz*., *Dendrocalamus membranaceus*, *D. strictus* and *Bambusa nutans*, to determine a combination of conditions ensuring the most regular, rapid and complete germination under laboratory conditions. The seeds were sown at different combinations of incubation temperatures (20, 25, 30, 35, 40°C and 20–30°C) and sowing media (top of paper, between paper and sand). Seeds were also sown in the presence and absence of light at 30°C on top of paper. The ideal conditions for testing seeds of all the three species were found to be 30°C, top of paper and preferably presence of light.

Key words: *Dendrocalamus membranaceus*; *D. strictus*; *Bambusa nutans*; seed; germination.

INTRODUCTION

It is indispensable to follow a standard pattern for testing seeds for germination in order to ensure uniformity and reproducibility of results. Testing under field conditions is normally unsatisfactory and, therefore, laboratory methods have been developed in which the external conditions are controlled to obtain most regular, rapid and complete germination of seeds [1]. The most important factors affecting germination under laboratory conditions are temperature, media and light, which need standardisation for each species. Temperature is one of the most critical factors and there is usually an optimal temperature below and above which germination is delayed or prevented. Soil is rarely used as a substrate for germination tests because each sample will vary greatly in physical, chemical and biological properties. Thus, the lack of reproducibility and difficulty in comparing tests of different seed lots renders it unsuitable. Artificial media are much more easily standardized and, hence, the International Seed Testing Association (ISTA) [2] recognized three germination media: top of paper (TP), between two layers of paper (BP) and sand. The effect of light on germination has been known to vary considerably with the seeds of different species. Some seeds germinate after being given a brief illumination while some are indifferent to the presence or absence of light during germination.

Such standardizations have been lacking for bamboo species probably due to rare availability of seeds. ISTA rules [1], though providing guidelines for testing few tropical tree species, do not mention bamboo species. The seeds of most bamboos have been reported to lose viability rapidly under ordinary storage conditions [3] and, therefore, require testing before sowing in nursery. The present paper reports optimum germination conditions for the seeds of three bamboo species, *viz*., *Dendrocalamus membranaceus*, *D. strictus* and *Bambusa nutans*, to obtain full germination potential of a seed lot.

MATERIALS AND METHODS

Seed

The seeds of *D. membranaceus* and *D. strictus* were collected from the campus of Forest Research Institute, Dehra Dun (Uttranchal, India) during 1992 and 1994, respectively, while the seeds of *B. nutans* were procured from Tropical Forest Research Institute, Jabalpur, MP. The seeds were collected during April from Sarguja area of MP where it flowered gregariously during 1994. The seeds stored at 5°C with around 5% moisture content were used for the following study, except in second experiment where seeds of *D. strictus* stored at 15°C were used.

Germination test

Constant temperatures of 20, 25, 30, 35, 40°C and alternating 20–30°C (16 h at 20°C and 8 h at 30°C) were used in combination with germination media TP, BP and sand. All the tests were carried out in four replications of 100 seeds each. Glass petridishes of 15 cm diameter lined with moist towel germination paper were used as TP. For BP, seeds were spread on moistened germination paper and rolled. Sterilized quartz sand as prescribed in ISTA rules, filled in enamelled trays, 45×30 cm in size, was used as a medium in which seeds were sown 1 cm deep. All the media were kept just moist throughout the test period using tap water. All the seeds sown as above were incubated in seed germinators set at specified temperatures with around 95% humidity. Light was provided for 8 h daily during the test period by cool fluorescent lights. Germination was recorded daily and a seed was counted as germinated when radicle and plumule attained at least 1 cm length and were free from visual fungal infection or deformation. In sand, a seed was considered as germinated when plumule attained at least 1 cm height above the sand surface. The test was terminated when there was no further germination. In the second experiment, the effect of light on germination was observed at 30°C on TP following the results of the above experiment. The germinators set at 30°C with and without light were used to investigate the effect of light. Care was taken to avoid light during recording of germination.

The mean germination time (MGT) was calculated as described by Bonner [4]. Treatments effect and interaction was analysed by ANOVA techniques after arc sin transformation of germination percentages.

RESULTS

The seeds of all the three species were found to be non-dormant and germinated readily within few days. The germination, in terms of percent as well as MGT was, however, significantly ($P < 0.01$) affected by incubation temperature, sowing media and their interaction as detailed below.

Effect of temperature on germination per cent

In *D. membranaceus* (Table 1), significant difference was observed in mean germination per cent at all the temperatures tested ($P < 0.05$), except between 35°C and 20–30°C, with 41.0 and 37.7%, respectively. The maximum mean germination of 46.2% was obtained at 30°C, while the minimum was recorded at 20°C (9.8%). In *D. strictus* (Table 2), the mean germination per cent at 30 and 25°C was maximum (84.3 and 82.6%, respectively) and significantly higher as compared to other temperatures ($P < 0.05$). This was closely followed by 35, 40 and 20–30°C with 78.3, 77.3 and 76.9% mean germination, respectively, and remained at par with one another. At 20°C the mean germination was reduced to 58.7%. In *B. nutans* (Table 3), at 30 and 35°C the mean germination was 54.6 and 57.6%, respectively, and was significantly higher ($P < 0.05$) than the rest of the temperatures. At 25, 20–30 and 40°C the mean germination ranged between

Table 1.
Effect of temperature and media on percent germination of *D. membranaceus* seeds

Temperature (°C)	Media			Mean
	TP	BP	Sand	
20	15.5 (23.2)	14.0 (22.0)	0.0 (00.0)	9.8 (15.0)
25	50.0 (45.0)	49.0 (44.4)	10.0 (18.4)	36.3 (36.0)
30	59.5 (50.5)	60.0 (50.8)	19.0 (25.9)	46.2 (42.4)
35	57.0 (49.0)	50.0 (45.0)	16.0 (16.4)	41.0 (39.2)
40	34.0 (35.6)	30.0 (33.2)	12.3 (20.5)	25.4 (29.8)
20–30	50.3 (45.2)	41.8 (40.3)	21.0 (27.3)	37.7 (37.6)
Mean	44.4 (41.4)	40.8 (39.3)	13.0 (19.3)	

Values in parentheses are arcsin transformed. CD at 5%: temperature 1.56; media 1.10; temperature × media 2.70.

Table 2.
Effect of temperature and media on percent germination of *D. strictus* seeds

Temperature (°C)	Media			Mean
	TP	BP	Sand	
20	69.5 (56.5)	71.5 (57.8)	35.0 (36.3)	58.7 (50.2)
25	87.5 (69.9)	83.0 (66.0)	77.3 (61.5)	82.6 (65.8)
30	87.5 (69.4)	86.5 (68.7)	78.8 (62.8)	84.3 (66.0)
35	85.5 (67.7)	86.0 (68.1)	63.5 (52.9)	78.3 (62.9)
40	86.5 (68.5)	84.5 (67.1)	61.0 (51.4)	77.3 (62.3)
20–30	82.3 (65.2)	78.3 (62.3)	70.3 (57.0)	76.9 (61.5)
Mean	83.1 (66.2)	81.6 (65.0)	64.3 (53.7)	

Values in parentheses are arcsin transformed. CD at 5%: temperature 2.57; media 1.82; temperature × media 4.45.

Table 3.
Effect of temperature and media on percent germination of *B. nutans* seeds

Temperature (°C)	Media			Mean
	TP	BP	Sand	
20	33.5 (35.4)	39.0 (38.6)	8.5 (16.9)	27.0 (30.3)
25	54.5 (47.6)	52.0 (46.2)	31.0 (33.8)	45.8 (42.5)
30	60.5 (51.1)	57.5 (49.3)	45.8 (42.6)	54.6 (47.7)
35	61.0 (51.4)	61.0 (51.4)	50.8 (45.5)	57.6 (49.4)
40	53.5 (47.3)	55.0 (47.9)	31.8 (34.3)	43.4 (41.2)
20–30	52.8 (46.6)	47.8 (43.7)	29.5 (32.9)	43.3 (41.1)
Mean	51.0 (45.6)	52.0 (46.2)	32.9 (34.3)	

Values in parentheses are arcsin transformed. CD at 5%: temperature 1.82; media 1.29; temperature × media 3.15.

45.8 and 43.3%, and remained at par with one another, while the minimum mean germination was recorded at 20°C (27.0%).

Effect of media on percent germination

In *D. membranaceus* (Table 1), TP gave maximum mean germination of 44.4% and was significantly different from other media ($P < 0.05$). BP followed this with 40.8% and the minimum 13.0% was recorded in sand. In *D. strictus* (Table 2), the mean germination of 83.1 and 81.6% was recorded on TP and in BP, respectively, and remained at par with each other. In sand, the mean germination was significantly reduced to 64.3%. Similarly, *B. nutans* (Table 3) showed significantly higher mean germination on TP (51%) and BP (52%) as compared to sand (32.9%).

Effect of temperature on mean germination time

In *D. membranaceus* (Table 4), the mean MGT was significantly different at all the temperatures ($P < 0.05$). The minimum was recorded at 30°C (15.32), while the maximum of 21.61 was recorded at 20°C. In *D. strictus* (Table 5), at 30 and 35°C the mean MGT was minimum (9.82 and 9.65, respectively) and at par with each other but significantly different from other temperatures ($P < 0.05$). The mean MGT was highest at 20°C (18.63). In *B. nutans* (Table 6), all the temperatures showed significant difference ($P < 0.05$) in mean MGT, except at 25 and 20–30°C, which were at par with each other with 16.60 and 16.80, respectively. At 35°C the mean MGT was lowest (13.27) followed by 30°C (14.16), while the maximum was obtained at 20°C (20.76).

Effect of media on mean germination time

In *D. membranaceus* (Table 4), significantly lowest mean MGT (16.27) was obtained in TP ($P < 0.05$), closely followed by BP (17.48) while maximum of

Table 4.
Effect of temperature and media on mean germination time (MGT) of *D. membranaceus* seeds

Temperature (°C)	Media			Mean
	TP	BP	Sand	
20	21.38	21.45	22.00	21.61
25	16.52	17.07	21.61	18.40
30	13.02	13.22	20.33	15.52
35	13.32	16.10	20.55	16.66
40	17.53	19.57	21.05	19.38
20–30	15.87	17.48	20.50	17.95
Mean	16.27	17.48	21.00	

CD at 5%: temperature 0.32; media 0.22; temperature × media 0.55.

Table 5.
Effect of temperature and media on mean germination time (MGT) of *D. strictus* seeds

Temperature (°C)	Media			Mean
	TP	BP	Sand	
20	17.14	18.08	20.69	18.63
25	9.82	8.93	14.52	11.09
30	7.44	7.74	13.79	9.65
35	7.48	7.50	14.47	9.82
40	8.01	8.04	14.12	10.05
20–30	9.87	9.63	14.87	11.46
Mean	9.96	9.99	15.41	

CD at 5%: temperature 0.44; media 0.31; temperature × media 0.75.

21.00 in sand. In *D. strictus* (Table 5), the lowest mean MGT, i.e., 9.96 and 9.99, was obtained at TP and BP, respectively, which remained at par, while the maximum was obtained in sand (15.41). A similar trend was observed in *B. nutans* (Table 6), where TP and BP showed minimum MGT of 14.80 and 14.69, respectively, as compared to sand (19.09).

Effect of light/dark on percent germination and MGT

In all the three species there was insignificant difference ($P > 0.05$) in percent germination of seeds sown under light and dark (data not shown). The germination was 57.0 and 56.0% in *D. membranaceus*, 61.5 and 64.0% in *D. strictus* and 55.0 and 54.5% in *B. nutans* in light and dark, respectively. Similarly, MGT was not significantly affected by the presence or absence of light in all the three species. The MGT was 10.97 and 11.18 in *D. membranaceus*, 9.15 and 9.00 in *D. strictus* and 10.17 and 10.30 in *B. nutans* in light and dark, respectively.

Table 6.
Effect of temperature and media on mean germination time (MGT) of *B. nutans* seeds

Temperature (°C)	Media			Mean
	TP	BP	Sand	
20	20.09	20.53	21.66	20.76
25	14.69	14.58	20.55	16.60
30	12.24	12.73	17.53	14.16
35	11.85	11.85	16.12	13.27
40	15.28	13.33	18.14	15.58
20–30	14.68	15.13	20.59	16.80
Mean	14.80	14.69	19.09	

CD at 5%: temperature 0.39; media 0.27; temperature × media 0.67.

DISCUSSION

The germination characteristics of seeds of bamboo species seem to be specific to a particular kind. All the three species under investigation exhibited more or less similar response with respect to incubation temperature, sowing media and presence or absence of light. Temperature is one of the most critical factors in the laboratory germination of seeds. This became evident in all the three species of bamboos, which showed 30°C as the ideal temperature for germination, though seeds of *D. strictus* and *B. nutans* germinated equally well at 25 and 35°C, respectively. As expected, it took minimum days to complete germination at 30°C as the MGT was minimum at this temperature, except *B. nutans*, which showed minimum at 35°C. At temperatures below or above 30°C, the germination was not only drastically delayed but also reduced in percentage with little exceptions. A critical temperature of 30°C has been shown to be ideal for several indigenous tropical tree species [5, 6]. Maximum germination at 30°C has also been reported in *D. strictus* [7] and *B. tulda* [8].

With respect to germination media, all three species of bamboos germinated equally well on TP and BP, except *D. strictus*, which performed best at TP. TP, however, was found to be the best as on this media seedlings could be evaluated more easily for abnormalities. Seedlings in BP remained somewhat whitish yellow due to lack of sufficient light, making it difficult to distinguish albino seedlings, which seems to be a characteristic of bamboo species. TP has been reported as a best media for germination of *B. tulda* seeds [8]. ISTA [2] also prescribed TP as the best media for germination of small seeds. In sand the germination was not only drastically slow but also reduced significantly.

The seeds of all the three species germinated equally well in dark and light. There was no significant difference in germination percent, as well as MGT in seeds sown in total darkness and in light ($P > 0.05$). However, a cycle of 8 h daily light seems to be essential for proper evaluation of seedlings for abnormality. Seedlings in dark remained whitish yellow making it again difficult to distinguish albino seedlings.

ISTA [2] also mentioned that seedlings grown in complete dark are etiolated and become more sensitive to attack by micro-organisms and it becomes difficult to detect chlorophyll deficiency.

Thus, the ideal conditions that could be used for determining germination capacity of a seed lot are 30°C, TP and preferably presence of light. Although the above study is based on a single seed lot of each species, it provides a base regarding testing conditions of bamboo seeds. The availability of different seed lots of a species of bamboo is a major constraint mainly due to long flowering intervals of several years.

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