Indian Forester, **143** (7) : 693-699, 2017
http://www.indianforester.co.in

ISSN No. 0019-4816 (Print)
ISSN No. 2321-094X (Online)

GROWTH PERFORMANCE EVALUATION OF TISSUE CULTURE RAISED BAMBOOS UNDER VARYING SPACE AND FERTILIZER LEVELS

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ABSTRACT

Bamboos occur naturally in tropical, subtropical and temperate regions around the world. Next to China, India is the major grower of bamboos and many species are cultivated in different agroclimatic regions. Due to the monocarpic nature of bamboos, propagules shortage is often realized through micropropagation approaches. However, field performance of growth traits need to be ascertained with reference to spacing intervals and fertilizer requirements. This study examined 5×5m and 6×6m spacing with different fertilizers like farmyard manure, chemical fertilizers and mixture of farmyard manure and chemical fertilizers for the three important bamboos species, i.e., *Dendrocalamus strictus*, *Pseudoxytenanthera stocksii* and *Bambusa bambos*. At the age seven years, it was observed that among three species *Bambusa bambos* exhibited greater height growth (16.19±2.31m) and culm diameter (79.94±7.32) whereas *Pseudoxytenanthera stocksii* produced higher number of culms (39.04±5.17) with the application of farmyard manure and chemical fertilizers. Spacing of 5×5m was suitable for *Dendrocalamus strictus* and *Pseudoxytenanthera stocksii* whereas, 6×6m was essential for *Bambusa bambos* for increased productivity. Further, yield potential can be enhanced with the application of farmyard manure and chemical fertilizers.

Key words: Tissue culture, Productivity, Field performance, Fertilizers.

Introduction

India is one of the major growers of bamboos next to China in the Asian region. Although about 130 species are reported in India, very few species are targeted for commercial utilization. Under the National Bamboo Mission (NBM) and National Mission on Bamboo Applications (NMBA), Government of India has promoted large scale cultivation of bamboos and prioritized 16 different species for cultivation to improve rural livelihood and promote entrepreneurships. Hence, demand for propagules has increased rapidly in the recent past. Several economically important species of bamboos have the flowering cycle varying from annual to 120 years and are monocarpic, thus making the seed availability irregular (Veller *et al.,* 2015). Hence, alternate methods like vegetative propagation of culm cuttings and rhizome planting and *in vitro* propagation were considered to be the options for planting stock production. Multiplication of bamboos through tissue culture methods with an aim to produce large number of plants is one of the major areas received the attention of nursery managers because of the possibilities of high number of propagules production in a short time with limited space and the size of propagules are smaller thus easy to handle.

Concerted efforts were made to develop tissue culture technology for large scale production, however the area under bamboo cultivation through tissue culture origin was the least and very few trails were laid to demonstrate the performance of tissue culture plants (Sood *et al*., 2002; Mishra *et al*., 2015; Gantait *et al*., 2016). Bamboos being a common component of forests and homesteads, their cultivation under farmland conditions demands demonstration trials to assess their growth performance of tissue culture raised plants and spacing and fertilizer requirements.

Field performance of tissue culture raised plants of agricultural and horticultural crops have been studied widely to adopt the technique for mass multiplication and to evaluate the agronomic traits in the field (Salvi *et al*., 2002; Devarumath *et al.,* 2007; Sandhu *et al*., 2009; Muniswamy *et al.,* 2015). In forest tree species only few like *Populus* (Sidhu and Dhillon, 2007), spruce (Grossnickle and Folk, 2007) were considered for field trials as most of the planting stock for large scale plantations are derived from hybrid material.

Field demonstrations trials are considered to be most important in perennial species to establish the consistency of the performance and subsequent promotion of the technique for wider adoption. Although

Suitable espacement and fertilizer application enhance the growth of tissue culture raised *Bambusa bambos*, *Dendrocalamus strictus* and *Pseudoxytenanthera stocksii*.

the cost of plants could be reduced, the capital cost for tissue culture is high and hence the knowledge on amenability of the species for tissue culture production is essential. Further, the demonstration trials boost the acceptance of tissue culture plants by the farmers, small scale industries and other planting agencies. Hence, to generate information on the field performance of the micro and macro propagated planting stock of selected economically important bamboos the Department of Biotechnology, Government of India has taken up a mission programme on field demonstration of quality planting material of bamboo in the tenth five year plan with a target of covering 1000 ha in 12 states (http://www.dst.gov.in/sites/default/files/rep-dep-bio.pdf). Under this mission, the present study was carried out to assess the field growth performance of plants produced through micropropagation for the species *Bambusa bambos*, *Dendrocalamus strictus* and *Pseudoxytenanthera stocksii* with different spacing and fertilizer treatments.

Material and Methods

Three species namely *Bambusa bambos*, *Dendrocalamus strictus* and *Pseudoxytenanthera stocksii* were selected to assess the effect of spacing and fertilizer applications. Micropropagation was carried out from the explants collected from selected seedlings of *Bambusa bambos* and *Dendrocalamus strictus* and selected clumps of *Pseudoxytenanthera stocksii*. The procedure for micropropagation and hardening of these species are mentioned elsewhere (Yasodha *et al.,* 2004). Plants with approximately 45-50 cm height were used for filed planting. Field trial was raised in a farm land used for cultivating turmeric and cotton located in Salem, Tamilnadu, India (11° 39' 18" N; 78° 9' 39" E) with irrigation facilities during August 2007. Field planting experiments consisted of two main plots for spacing trials and 4 subplots for fertilizer trials for each species. The spacing (S) and fertilizer (F) trial consists of following treatments: S_1F_1, S_1F_2, S_1F_3, S_1F_4, S_2F_1, S_2F_2, S_2F_3 and S_2F_4

S_1 - 5×5m

S_2 - 6×6m

F_1 - No fertilizer

F_2 - 10kg farm yard manure

F_3 - 50g urea+50g super phosphate+25kg muriate of potash

F_4 - 10kg of farmyard manure+50g urea+50g super phosphate+25kg muriate of potash.

Planting for each species was carried out in a completely randomised block design with three replications. The pit size for planting was 45×45×45 cm and each pit was filled with the above mentioned fertilizer dosage. Each treatment in a replication received 36 plants. The fertilizer dosage was repeated once in a year for initial three years. The field was flood irrigated once in 30 days throughout the period. Data on number of culms per clump, height of the tallest culm and diameter of the culm (at 6th inter node) were recorded in August 2014. Survival of the plants was assessed in three months period and the dead plants were replaced within six months. The data were analysed using the SPSS ver. 22 and analysis of variance (ANOVA) was performed to test the effect of species, space and fertilizer application on various growth parameters over the years. Post hoc multiple comparisons of means were carried out for individual species.

Results and Discussion

Advantages of the tissue culture derived bamboo plants and their superior performance in the field were documented for few species (Agnihotri *et al.*, 2009; Singh *et al.,* 2013). Field performance of tissue culture produced rubber clones showed that they are equivalent or better in growth and should be considered for increase in productivity (Dibi *et al.,* 2010). Several grey literatures are available about the spacing requirements of bamboos, but no specific study addressed the spacing and fertilizer treatments on growth of tissue culture raised plants of bamboos. Generally, use of fertilizers in bamboos are not common, however, under agroforestry conditions, to attain the economically benefiting growth performance, application of fertilizers are suggested (Kittur *et al.,* 2015). In the present study, investigations were carried out to estimate the effects of spacing and fertilizers and their combinations in three commonly cultivated bamboo species namely *Dendrocalamus strictus*, *Pseudoxytenanthera stocksii* and *Bambusa bambos* raised through axillary bud proliferation. After field planting, plants that produced new shoots within three months of planting were considered to be established and the plants dried off were replaced. Survival rate was estimated to be 97 per cent for *Dendrocalamus strictus* and 98 per cent for *Pseudoxytenanthera stocksii* and *Bambusa bambos* during August 2008. Period of transplanting *in vitro* raised plants to field has direct influence on the survival rate, wherein July to August being peak monsoon months higher survival rate was recorded in many field studies and the survival percentage was ranged from 70-100 (Singh *et al*., 2013). In bamboos, generally the culms are harvested from 5-6 year onwards and thereafter more than three year old culms are felled annually or biannually depending on their availability. In this study, three growth traits namely height of the tallest culm, diameter of culm and number of culms produced were measured, which are directly relevant to the yield of bamboos.

There was a highly significant (ANOVA; P<0.05) effect for height, number of culms and culm diameter expressed among species, space and fertilizers individually and species with space interaction (Table 1). No significant interaction was observed for space and fertilizer, species and fertilizer and species-space-fertilizer effect. Further, culm diameter also did not show significant influence with space. Within the species, *Dendrocalamus strictus* showed significant effect for height (P<0.001) and culm diameter (P=0.02) (Table 2). Similarly space had an effect on number of culms produced. Space and fertilizer interaction effect on the three traits was not significant among the three species studied. Effect of fertilizer was pronounced (P<0.01) in *Pseudoxytenanthera stocksii* but spacing had no effect. *Bambusa bambos* had highly significant (P<0.001) effect of space on growth, whereas fertilizer influened only height growth (P=0.002). Under agroforestry conditions, spacing of 8×8m was recommended for *Dendrocalamus strictus*, which was intercropped with turmeric in Kerala for sustainable management of better crop and bamboo performance (Kittur *et al*., 2015). In the tissue culture raised *Dendrocalamus hamiltonii* 6×6m was practiced by Sood *et al.* (2002) and in *Bambusa balcooa* 3×3m was practiced by Gantait *et al.* (2016). Such variations in spacing may be specific for species, and growing environments like pure plantations or agroforestry systems (Banerjee *et al*., 2009).

Among the three bamboo species, significant variations were observed for height, number of culms and culm diameter (Fig. 1). Height growth of the culms in *Bambusa bambos* was higher (16.18m) followed by *Dendrocalamus strcitus* (11m) and *Pseudoxytenanthera stocksii* (10.3m). Number of culms (39) produced in *Pseudoxytenanthera stocksii* was larger in seven years of age, where as *Dendrocalamus strictus* produced 25 culms and *Bambusa bambos* generated 30 culms. The fertilizer treatments were compared across the species and found that the treatments F_2 (10kg farmyard manure) and F_4 (10 kg farmyard manure + 50g urea + 50g super phosphate + 25kg muriate of potash) always showed higher growth in terms of height of the culm, number of culms and culm diameter (Fig. 2). In F_3 treatment, the growth performance was similar to control except for height, where the control was comparatively lower in height. Tissue culture raised plants of *Bambusa balcooa* received 10kg of farmyard manure along with chemical fertilizers and in the second year 8.68m height growth was recorded (Gantait *et al.,* 2016).

Table 1: ANOVA of the effects of species, space and fertilizers and their interaction on growth traits of bamboo species.

Effect/Growth parameters	Species		Space		Fert		Space × Species		Space × Fert		Species ×Fert		Space × Species × Fert	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Height (m)	229.28	<0.001	24.72	<0.001	30.31	<0.001	20.23	<0.001	0.66	0.577	1.10	0.372	0.49	0.806
Number of culms	85.32	<0.001	35.04	<0.001	4.10	0.011	9.27	<0.001	0.19	0.897	1.57	0.176	0.51	0.793
Culm diameter (mm)	376.67	<0.001	3.49	0.068	13.18	<0.001	17.17	<0.001	0.71	0.546	1.82	0.114	0.60	0.728

Table 2: ANOVA of the effects of space and fertilizers on growth traits of each species.

Fixed effect	Height (m)			Number of culms			Culm diameter (mm)		
	df	F	P	df	F	P	df	F	P
D.strictus									
Space	1	1.472	0.243	1	6.38	0.022	1	0.987	0.335
Fertilizer	3	16.798	<0.001	3	0.755	0.535	3	4.271	0.021
Space × Fertilizer	3	0.113	0.144	3	0.951	0.932	3	0.113	0.144
Error	16			16			16		
P.stocksii									
Space	1	0.356	0.559	1	1.736	0.206	1	3.314	0.087
Fertilizer	3	9.135	<0.001	3	5.202	0.011	3	13.316	<0.001
Space × Fertilizer	3	0.695	0.568	3	0.599	0.625	3	0.803	0.511
Error	16			16			16		
B.bambos									
Space	1	46.925	<0.001	1	38.790	<0.001	1	35.625	<0.001
Fertilizer	3	7.827	0.002	3	0.431	0.733	3	2.514	0.095
Space × Fertilizer	3	0.769	0.528	3	0.344	0.794	3	1.234	0.330
Error	16			16			16		

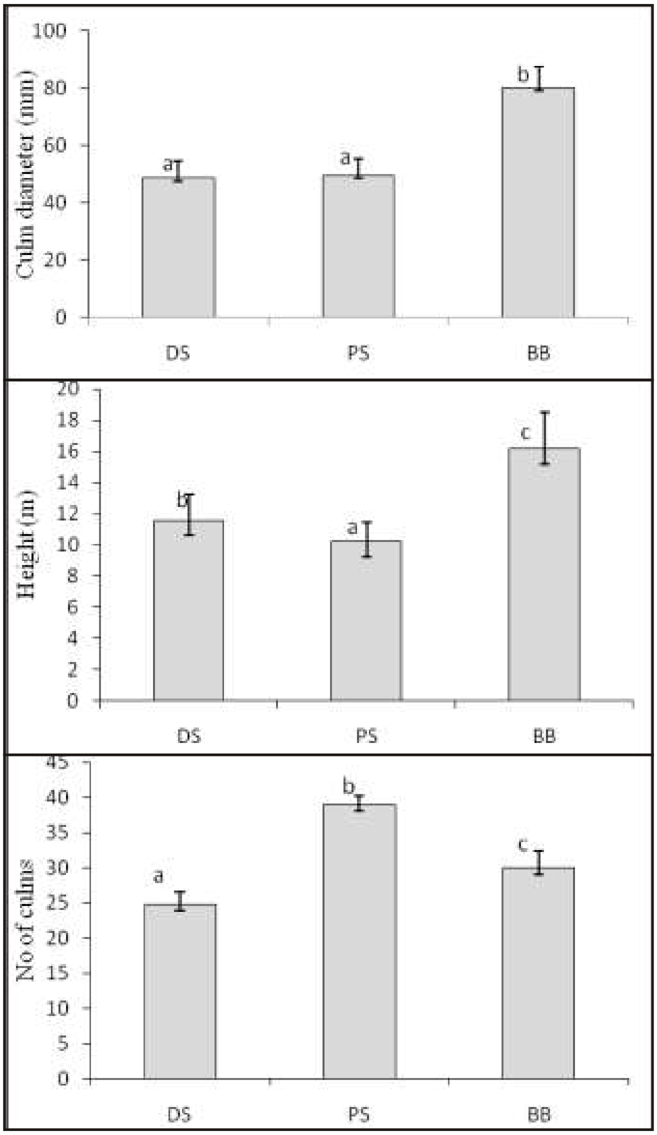

Fig. 1: Growth variations in height(m), number of culms produced and culm diameter(mm) of the three bamboo species at the age of seven years. Error bars indicate the standard error of the mean (n = 24); columns not sharing the same letter indicate significant differences according to the ANOVA in SPSS (P = 0.05). DS - *Dendrocalamus strictus;* PS – *Pseudoxytenanthera stocksii;* BB – *Bambusa bambos.*

Effect of spacing among the species were analysed with paired t-test and the results are displayed in box plots (Fig. 3). Spacing had no effect on the height and culm diameter of *Dendrocalamus strictus* and *Pseudoxytenanthera stocksii* (Fig. 3a and 3g). However, significant effect was observed in *Dendrocalamus strictus*, where the mean number of culms produced was 23.4 with 6×6m spacing and mean number of culms produced was 26.3 with 5×5m spacing (Fig. 3d). No significant difference observed among the spacing treatments in *Pseudoxytenanthera stocksii* for all the traits analysed (Fig. 3b, 3e and 3h). *Bambusa bambos* showed significant variations for height

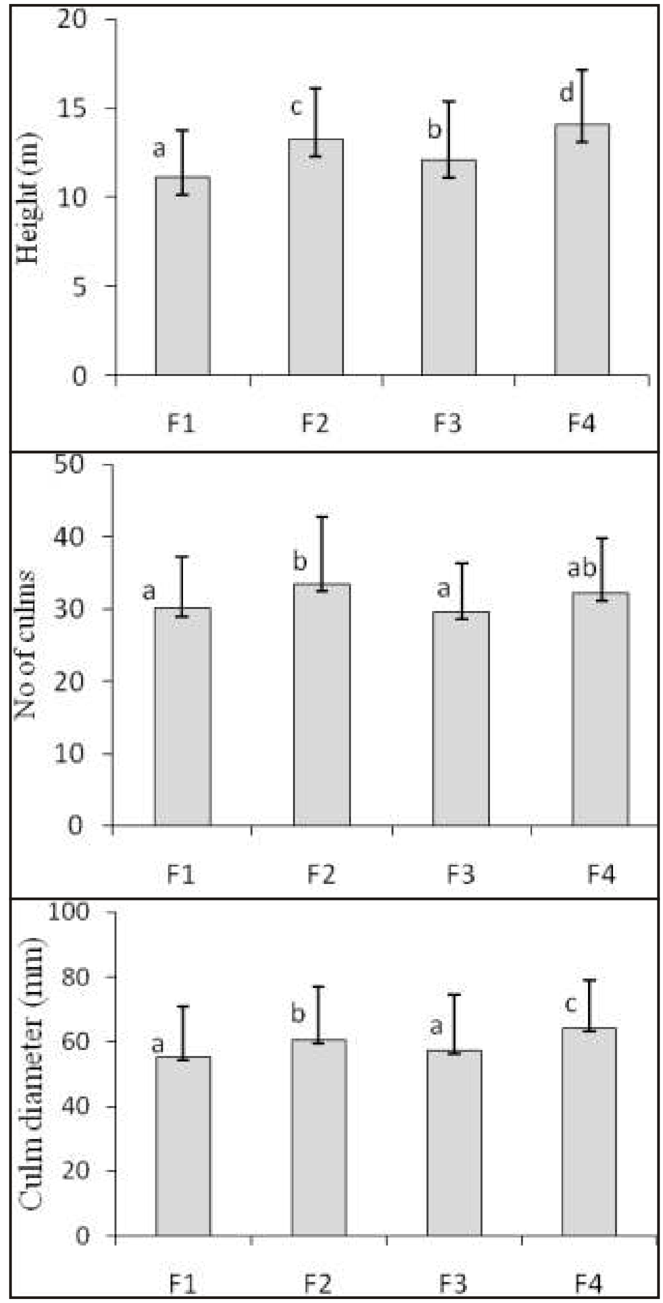

Fig. 2: Effect of four fertilizer treatments (F1, F2, F3, F4) on height(m), number of culms produced and culm diameter(mm) in bamboos. Error bars indicate the standard error of the mean (n = 18); columns not sharing the same letter indicate significant differences according to the ANOVA in SPSS (P = 0.05).

and culm diameter as the spacing effect but number of culms produced were not affected by the spacing.

The genotype means for height growth, number of culms and culm diameter for the three bamboo species tested for spacing and fertilizer treatments and their interaction at the age of seven years are presented in Table 3, 4 and 5. Height growth was higher (13.26m) in *Dendrocalamus strictus* in F_4 treatment whereas

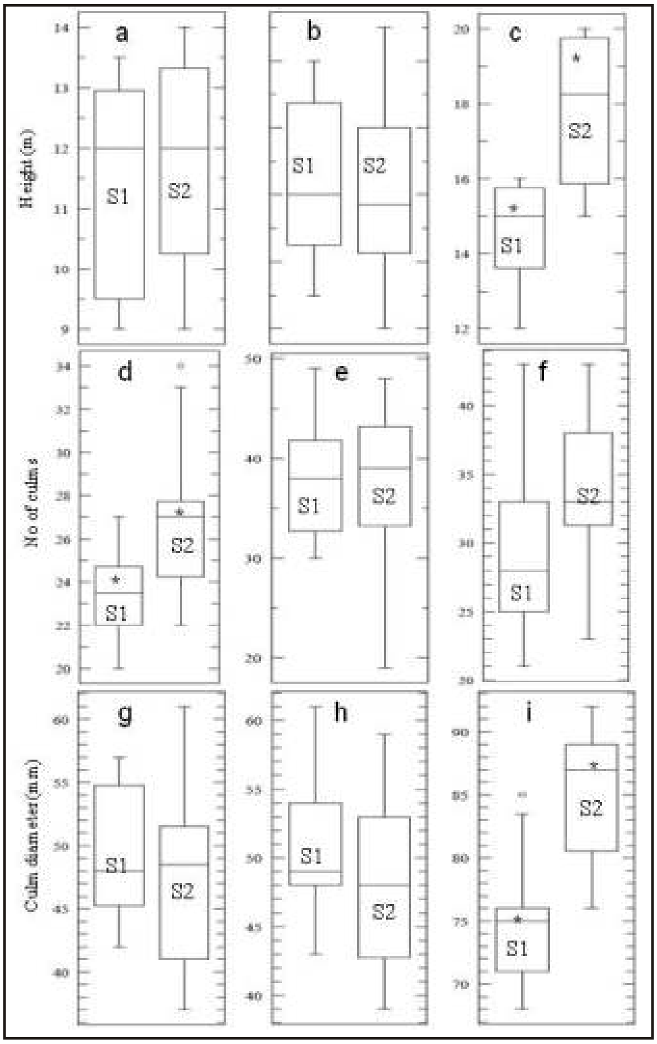

Fig. 3: Box plot of the variation for height, number of culms produced and culm diameter in *D. strictus* (a, d, g), *P.stocksii* (b, e, h) and *Bambusa bambos* (c, f, i) under 5m x 5m spacing (S1) and 6m x 6m spacing (S2)treatments,. The ends of the box represent the first and third quartiles and the middle line represents the median. The error bars indicate 1.5-fold the interquartile range. The asterisks indicate significant differences for height in the S1 and S2 treatment. The significance (< 0.05) was tested using the paired t-test.

Pseudoxytenanthera stocksii and *Bambusa bambos* had tallest culms with the height of 11.42m and 17.67m respectively in F_2 treatment (Fig. 4). Application of chemical fertilizers (NPK) significantly influenced culm number and diameter but not culm height in the micpropagated bamboos such as *Bambusa nutans*, *Bambusa tulda* and *Dendrocalamus asper* (Mishra *et al*., 2015).

In agroforestry systems, seven year old *Dendrocalamus strictus* produced culms of height 7.3 to 9.1m with number of culms 48 to130 without fertilizer application till seven years (Kittur *et al*., 2015), whereas in the present study, the mean height of 11.57m with 25 number of culms was recorded in the seventh year. Results of this study indicate the influence of fertilizer on height growth of bamboos. Likewise, application of NPK fertilizer showed increase in culm production but the diameter of the culms was reduced (Piouceau *et al*., 2015).

The trial was established in a farmer's field and the mature culms of *Pseudoxytenanthera stocksii* and

Table 3: Effect of spacing and fertilizer treatments on the growth of *D. strictus* (F_1-F_4; represents fertilizer applications and S_1F_1, S_2F_1.... represents spacing of 5×5m (S_1) and 6×6m (S_2) under different fertilizer treatments).

Treatments	Mean Height (m) ±SD	Mean No of culms ±SD	Mean Culm diameter (mm) ±SD
S_1F_1	9.77±0.93	23.00±1.00	44.67±2.50
S_1F_2	12.33±0.58	24.00±1.00	54.67±2.52
S_1F_3	10.17±1.61	22.33±2.50	46.00±1.00
S_1F_4	13.10±0.36	24.33±2.52	53.00±3.61
Mean	11.34±1.70	23.42±1.83	49.58±5.02
S_2F_1	10.00±1.00	26.67±1.53	42.00±4.58
S_2F_2	12.83±1.04	27.67±6.03	51.00±12.49
S_2F_3	11.00±1.00	24.67±2.54	46.33±4.73
S_2F_4	13.43±0.60	26.33±2.08	50.33±1.53
Mean	11.82±1.64	26.33±3.20	47.42±7.13
F_1	9.88±0.87	24.83±2.31	43.33±3.61
F_2	12.58±0.80	25.83±4.35	52.83±8.30
F_3	10.58±1.28	23.5±2.58	46.16±3.06
F_4	13.26±0.48	25.33±2.33	51.66±2.87
Grand Mean	11.57±1.65	24.87±2.95	48.5±6.12

Table 4: Effect of spacing and fertilizer treatments on the growth of *P. stocksii*. (F_1-F_4; represents fertilizer applications and S_1F_1, S_{2F1}.... represents spacing of 5×5m (S_1) and 6×6m (S_2) under different fertilizer treatments).

Treatments	Mean Height (m) ±SD	Mean No of culms ±SD	Mean Culm diameter (mm) ±SD
S_1F_1	9.17±0.76	35.00±4.36	48.33±3.51
S_1F_2	11.00±1.00	42.67±7.09	48.33±5.51
S_1F_3	10.00±1.00	33.33±4.16	48.67±1.15
S_1F_4	11.17±1.04	40.67±1.53	58.00±3.61
Mean	10.33±1.17	37.92±5.70	50.83±5.39
S_2F_1	9.08±0.38	38.67±4.51	44.00±4.58
S_2F_2	10.67±0.58	45.67±2.08	48.67±1.15
S_2F_3	9.07±0.93	37.33±4.73	43.33±4.16
S_2F_4	11.67±1.04	39.00±2.00	56.67±2.52
Mean	10.12±1.33	40.17±4.55	48.17±6.26
F_1	9.13±0.54	36.83±4.45	46.17±4.36
F_2	10.83±0.75	44.17±4.96	48.50±3.56
F_3	9.53±1.00	35.33±4.55	46.00±4.00
F_4	11.42±0.97	39.83±1.83	57.33±2.88
Grand Mean	10.23±1.23	39.04±5.17	49.50±5.88

Table 5: Effect of spacing and fertilizer treatments on the growth of *B.bambos*. (F_1-F_4; represents fertilizer applications and S_1F_1, S_2F_1.... represents spacing of 5×5m (S_1) and 6×6m (S_2) under different fertilizer treatments).

Treatments	Mean Height (m) ±SD	Mean No of culms ±SD	Mean Culm diameter (mm) ±SD
S_1F_1	13±1.00	22.66±4.04	72.33±3.78
S_1F_2	14.5±0.86	26.33±3.05	73.33±3.05
S_1F_3	15±1.00	25±4	76.33±8.50
S_1F_4	15.66±0.57	24.67±2.51	76.16±1.60
Mean	14.54±1.26	24.67±3.28	74.54±4.60
S_2F_1	15.83±1.04	34.33±5.86	80.00±2.00
S_2F_2	18.50±0.50	34.67±3.06	88.00±2.65
S_2F_3	17.33±2.52	35.00±4.58	83.33±6.66
S_2F_4	19.67±0.58	37.67±5.51	90.00±1.73
Mean	17.83±1.91	35.42±4.38	85.33±5.23
F_1	14.42±1.80	28.50±7.82	76.17±5.00
F_2	16.50±2.28	30.50±5.32	80.67±8.43
F_3	16.17±2.14	30.00±6.69	79.83±7.83
F_4	17.67±2.25	31.17±8.08	83.08±7.72
Grand Mean	16.19±2.31	30.04±6.66	79.94±7.32

Bambusa bambos were removed for commercial purpose by selective felling from fifth year onwards. In the case of *Dendrocalamus strictus*, no culms were removed from the clumps due to difficulty in extraction of culms. Fig. 4 shows the prominent central lumen in *Bambusa bambos* and almost no lumen in *Pseudoxytenanthera stocksii* at the age of seven years. Similar to conventionally raised bamboo plants, tissue culture raised plants were also produced the harvestable culms from fifth year onwards in *Dendrocalamus hamiltonii* (Sood *et al*., 2002). In the present study, the culms from *Bambusa bambos* and *Pseudoxytenanthera stocksii* were cut and used for making articles like baskets, mats and furniture. Although good growth was recorded, the species selected in this study are clumping or sympodial bamboos, posing difficulties in extraction of culms at maturity because of the non-availability of trained manpower in the locality.

Fig. 4: Growth performance of micropropagated plants of bamboo species (seven years old). a. *D.strictus*; b. *B.bambos* c. *P.stocksii*; d. *D.strcitus* clumps after selective removal of mature culms; e and f. Basal region of culms of *B.bambos* and *P.stocksii* showing the lumen.

Conclusion

The species with slender culms such as *Pseudoxytenanthera stocksii* and *Dendrocalamus strictus* exhibited better growth with the 5×5m espacement. While species like *Bambusa bambos* with larger culm size required planting espacement of 6×6m. Further, it was evident from this study that the chemical fertilizers with farmyard manure enhanced the growth of bamboos instead of applying chemical fertilizers alone or without fertilizers. No interaction effect of spacing and fertilizer on the growth was recorded in these bamboos.

Acknowledgments

The authors are grateful to the Department of Biotechnology, Government of India for the financial support.

विभिन्न अन्तराल एवं उर्वरक स्तरों के अन्तर्गत ऊतक संवर्धन से उगाए गए बाँसों का वृद्धि प्रदर्शन मूल्यांकन

वी.के.डब्ल्यू. बाजपेयी, एम. गणेशन एवं आर. यशोधा

सारांश

बाँस विश्वभर में उष्णकटिबंधीय, उप उष्णकटिबंधीय एवं शीतोष्ण क्षेत्रों में प्राकृतिक रूप में पाया जाता है। चीन के बाद भारत बाँसों का प्रमुख उत्पादक है तथा विभिन्न कृषि जलवायवीय क्षेत्रों में अनेकों प्रजातियों की खेती की जाती है। बाँसों की एकाण्डपी प्रकृति के कारण सूक्ष्मप्रवर्धन एप्रोचों के जरिए प्रवर्ध्य कमी प्रायः महसूस की गई। तथापि, अन्तरालन एवं उर्वरक आवश्यकताओं के संदर्भ में वृद्धि विशेषकों के क्षेत्र प्रदर्शन का पता लगाने की आवश्यकता है। इस अध्ययन में तीन महत्वपूर्ण बाँस प्रजातियों, यथा – *डेन्ड्रोकैलामस स्ट्रिक्टस, स्यूडोऑक्सीटीनेन्थीरा स्टॉकी* और *बैम्बूसा बैम्बोस* के

लिए फार्मयार्ड खाद, रासायनिक उर्वरक और फार्मयार्ड खाद एवं रासायनिक उर्वरकों के मिश्रण जैसे विभिन्न उर्वरकों के साथ 5 x 5 मी. और 6 x 6 मी. अन्तरालों की जांच की गई। सात साल की आयु में यह प्रेक्षित किया गया कि तीन प्रजातियों में बैम्बूसा बैम्बोस ने अत्यधिक ऊँचाई वृद्धि (16.19 $\pm$ 2.31 मी.) और नाल व्यास (79.94 $\pm$ 7.32) का प्रदर्शन किया जबकि *स्यूडोऑक्सीटीनेन्थीरा स्टॉकी* ने फार्मयार्ड खाद और रासायनिक उर्वरकों के उपयोग के साथ नालों की उच्चतम संख्या (39.04 $\pm$ 5.17) में उत्पादन किया। *डेन्ड्रोकैलामस स्ट्रिक्टस* एवं *स्यूडोऑक्सीटीनेन्थीरा स्टॉकी* के लिए 5 x 5 मी. का अन्तराल उपयुक्त था जबकि वर्धित उत्पादकता के लिए बैम्बूसा बैम्बोस हेतु 6 x 6 मी. आवश्यक है। इसके अलावा, उत्पादन क्षमता को फार्मयार्ड खाद और रासायनिक उर्वरकों के उपयोग के साथ बढ़ाया जा सकता है।

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