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Effect of different concentrations of BAP on *In vitro* shoot multiplication of bamboo

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Abstract
The present experiment was laid out in completely randomized design with 10 different treatments and 3 replications. The 10 different treatments involves use of different concentrations of cytokinin 6-banzylaminopurine (BAP) in Murashinge and skoog media ranging from 0 to 9 mg/lt. The experiment conducted successfully established the *in vitro* culture of nodal explant of green bamboo *Bambusa Balcooa.* The sterilization protocol was also successful to avoid contamination. Use of bavisitin, tween 20 and ethanol is enough for disinfection of explant. It was observed that the BAP concatenation of 4mg/lt and 5 mg/lt is best for initiation of shoot bud i.e. breaking dormancy of shoot bud compared to all other treatments. Moreover it was observed that BAP concentration 4mg/lt induces most number of shoots per explant. This treatment T_5 is also useful for increasing shoot length and number of leaves per explant after 21 days cycle.

Keywords: bamboo, BAP, *in vitro* culture, shooting, plant tissue culture

1. Introduction
Bamboo species have long history as a versatile and widely used renewable resources more than 4000 traditional uses and 1500 commercial applications have been identified for Bamboo including construction, paper, textile and board. Bamboo shoots are rich in various nutrients, minerals and also contains lignin and phenolic acids which have anti-cancer, antibacterial and antifungal activity. (Bhatt *et al.* 2005 David and Nirmala. 2007) [5].
The most important use of bamboo is as a raw material in pulp, about 1.9 million tones used by pulp industries. About 4.9 million metric tonnes are being presently utilized for paper making (Varmah and Pant, 1981). In the lives of rural poor and rural industries the bamboos occupy a special place, especially in Asia (Anonymous, 1978). Young new shoots of bamboo are eaten in Asia. In India bamboo is used for making paper, since it provides good quality paper pulp (Mehra S.P. and Mehra L.K. 2007). Bamboo can be used as materials for house construction, daily sundry goods, agricultural and fisheries tools, and crafting material (Chang and Ho 1997) [6]. Green bamboo is the strongest and fastest growing perennial grass species and is unique with complex branching pattern, woody culms and gregarious, monocarpic flowering plant under the family of poaceae.
Conventionally, bamboo are propagated through seed, clump division, rhizome, offset and clum cutting. However, gregarious flowering at long intervals followed by the death of clump (Austin and Marchesini 2012), short viability of seed (Bereket 2008), presence of diseases and some pests (Singh *et al.* 2013) are limiting factors to use seeds as valuable source of propagation. Vegetative propagation methods have limitations for mass propagation i.e. Propagules are different to extract, bulky to transport and planting material are insufficient in number for large -scale plantation (Kassahun 2003; Mudoi *et al.* 2013). Seasonal dependence, low survival rate and limited rooting of the propagules are other limitations (Singh *et al.*2013). Micropropagation is a process of vegetative growth and multiplication from plants tissue or seed. It is carried out in aseptic and various plant tissue culture technique. For large scale production, efficiency of axillary shoot proliferation is safe with less risk of aberration for rapid shoot induction and rooting. (Geilis and Oprins, 1998) [8].
Schroder in 2010 proposed that green bamboo is one of the largest bamboo species, it has high ornamental value, this bamboo species also produces large amount of biomass. Geilis and oprins in 1998 [8] said that for large scale production efficiency of axillary shoot proliferation method is safe with less risk of aberration and basic research allows identifying endogenous and exogenous factors for rapid shoot induction and rooting. Hence by considering above points in view an experiment entitles "Effect of different concentration of BAP on *in vitro*

shoot multiplication of Bamboo“ was conducted MGM College of Agricultural Biotechnology, Gandheli, Aurangab.

2. Materials and Methods

The details of various materials and experimental methods adopted during the course of present investigation are narrated in this chapter.

2.1 Experimental site

All experimental studies were conducted in Department of Biochemistry and molecular biology, MGM College of Agricultural Biotechnology, Gandheli, Aurangabad.

2.2 Experimental details

This work was under taken to study effect of different concentrations of 6-benzyladenine (BAP) on *in vitro* shoot proliferation of Bambusa balcooa. The nodal explant of bamboo is used for inocualtion in MS media supplemented with BAP growth hormone. The cytokinine at is responsible for *in vitro* shooting of bamboo explant.

2.3 Treatment details

2.3.1 Statistical Design: Completely Randomized Design

2.3.2 Total Number of treatments: 10 (Different concentrations of BAP 0 to 9 mg/lt)

2.3.3 Total Number of Replications: 03

Table 1: Different concentrations of BAP used in MS media

Sr. No.	Symbol	MS Media +Concentrations of BAP (mg/lit)
1	T_0	0
2	T_1	1.0
3	T_2	2.0
4	T_3	3.0
5	T_4	4.0
6	T_5	5.0
7	T_6	6.0
8	T_7	7.0
9	T_8	8.0
10	T_9	9.0

2.4 Media preparation

The Murashige and Skoog basal media was prepared by adding various components from stock solution in autoclaved distilled water. The concentration of 6-Benzyladeninepurine (BAP) was ranged from 0 to 9 mg/l. These stock of 10 mg/lit of BAP was prepared by dissolving powder in 1 N sodium hydroxide. The concentration of Sucrose was 30 gm/ lit and clarigel at concentration of 3g/lit was used for solidification. The pH of the medium was adjusted to 5.8 before addition of clerigel. The 40 ml media was poured into each culture bottles. They were then autoclaved at 121℃ for 20 minutes at 15 psi pressure and transferred to culture room where they were kept under dark for 72 hours before inoculation (Murashige and Skoog 1962) [22].

2.5 Explant collection

The explant used to initiate aseptic culture were axillary shoots 2.5 to 3.0 cm in length. They were collected from MGM Gandheli campus, Aurarngabad. The plan age was 2 to 3 years. The mother plant of Bambusa Balcooa was healthy and disease free. The explant of suitable size was cut out from mother plant and brought inside laboratory for further processing.

2.6 Surface sterilization of explant

The explant was obtained from mother plant present in open field. hence sterilization is of at most important to avoid the growth of unwanted microorganism under *in vitro* condition. These explants were collected from healthy mother plants. Then these explants are washed with RO water to remove dirt. Then the explants were washed with sterile distilled water for three times. After this the explants were transferred in Laminar Air Flow Cabinet for further sterilization. Inside Laminar Air Flow Cabinet these explants were again washed with bavistin for 10 minutes followed by tween -20 for 5 minutes followed by three washes of autoclaved distilled water. After the explants were treated with 70% ethanol for 30 seconds and then rinsed with sterile distilled water for three times. These explants are suitable for inoculation (Yuan *et.al.* 2009).

2.7 Inoculation of explant

The nodal explant was used for inoculation. Before inocualtion the ends of nodal explant were cut using blade. This properly trimmed explant is then inoculated in upright position inside MS media.

2.8 Culture condition

After inoculation of explants these culture bottles were capped and sealed with parafilm strips. These culture bottles were transferred to culture room. All the cultures were incubated in culture room at 25 ± 2ºC, with a relative humidity of 50-60 percent and photoperoid of 16 hours light.

2.9 Observations and analysis

The inoculated explant were observed for development of contamination for 21 days. Then meanwhile days required for initiation of shoot were counted. Number of shoots per explant were also recorded. Moreover the explant having fully grown shoots were used for measuring shoot length. Number of leaves per explant were counted. The quantitative data obtained on various observations was analyzed by “Analysis of variance (ANOVA)” method (Panse and Sukhatme, 1967).

Result and Discussion

3.1 Surface Sterilization

The surface sterilization of explants is very essential for successful establishment of plant tissue culture. The protocol for surface sterilization was successfully established. Explants are washed with RO water to remove dirt. Then the explants were washed with sterile distilled water for three times. After this the explants were transferred in Laminar Air Flow Cabinet for further sterilization. Inside Laminar Air Flow Cabinet these explants were again washed with bavistin for 10 minutes followed by tween -20 for 5 minutes followed by three washed of autoclaved distilled water. After the explants were treated with 70% ethanol for 30 seconds and then rinsed with sterile distilled water for three times. It was found that the explants reminded sterile under *in vitro* condition without any sign of contamination for 21 days.

3.2 Number of shoots per explants

Data of average number shoots per explants as influenced by different concentration of growth hormone 6- benzyl adenine purine is given in table below

Table 2: Average Number of shoots per explant

Treatment (Concentration of BAP)	Average Number of shoots per explants
T_1(0.0mg/lit)	0.33
T_2(1.0mg/lit)	2.33
T_3(2.0mg/lit)	3.00
T_4(3.0mg/lit)	3.67
T_5(4.0mg/lit)	7.67
T_6(5.0mg/lit)	3.33
T_7(6.0mg/lit)	3.67
T_8(7.0mg/lit)	4.00
T_9(8.0mg/lit)	2.33
T10 (9.0 mg/lt)	0.67
C.D.(0.01)	1.749

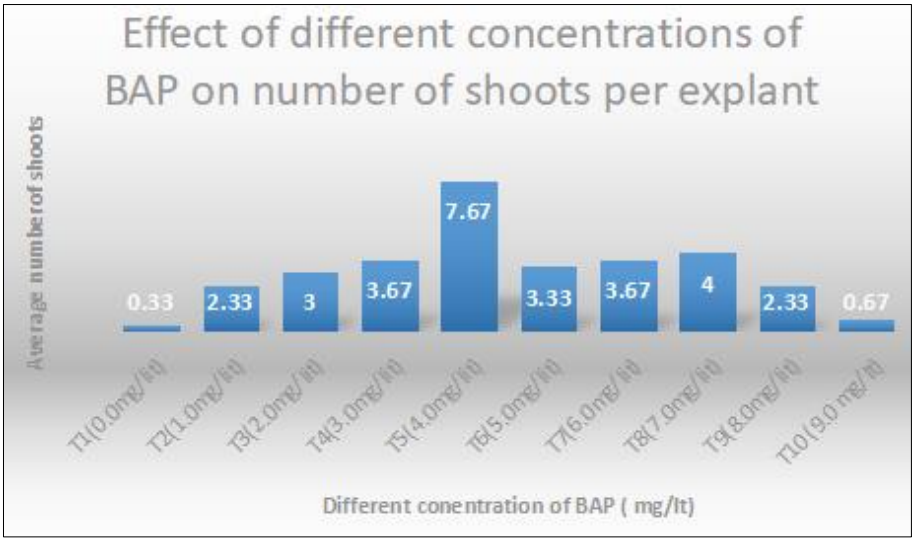

Fig 1: Effect of different concentration of BAP on shoot per explant

Data presented in table 2 and depicted in Fig. 1 revealed that the number of shoots emerged from explant is significantly influenced by different concentration of BAP. Highest number of shoots was found with treatment T_5 (4mg/lt BAP) which is significantly superior over rest of other treatments. Treatment T7, T8, T6, T9, T4 and T2 are at par with each other. From above data it is found that BAP when is beneficial for increasing number of shoots of explant. The treatment T1 and T10 are not useful for shoot bud development.

3.3 Average shoot length

Data of average shoot length as influenced by different concentration of growth hormone BAP is given in table below

Table 3: Average shoot length (cm)

Treatment (Concentration of BAP)	Average shoots length (cm)
T_1(0.0mg/lit)	0.33
T_2(1.0mg/lit)	2.67
T_3(2.0mg/lit)	4.67
T_4(3.0mg/lit)	5.33
T_5(4.0mg/lit)	7.67
T_6(5.0mg/lit)	7.67
T_7(6.0mg/lit)	6.00
T_8(7.0mg/lit)	6.67
T_9(8.0mg/lit)	6.33
T_{10} (9.0mg/lt)	6.67
C.D.(0.01)	1.469

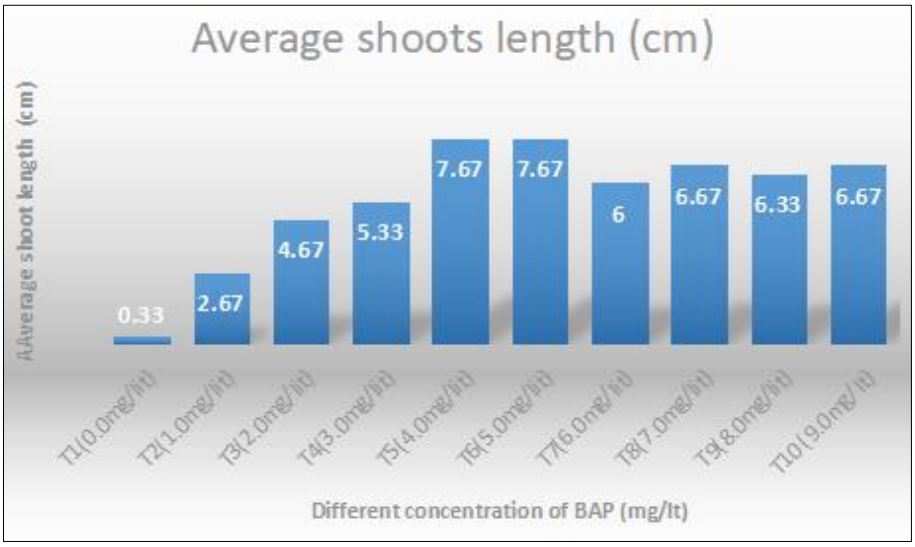

Fig 2: Average shoot length

Data presented in table 3 and figure 2 clearly indicates that the average shoot length is significantly influenced by different concentration of BAP. The highest shoot length was obtained in treatment T 5 and T6 which are superior over treatment T7. These treatment (T5 and T6) however are at par with T8, T9, T10. The treatment T8, T9, T10, T5 and T6 are superior over treatment T1, T2, T3 and T4.

3.4 Days required for shoot initiation

Data of average number of days required for initiation of shoots as influenced by different concentration of growth hormone BAP is given in table below.

Table 4: Average number of days required for shoot initiation

Treatment (Concentration of BAP)	Average Days required
T_1(0.0mg/lit)	18.67
T_2(1.0mg/lit)	8.33
T_3(2.0mg/lit)	5.67
T_4(3.0mg/lit)	4.67
T_5(4.0mg/lit)	2.67
T_6(5.0mg/lit)	3.67
T_7(6.0mg/lit)	5.67
T_8(7.0mg/lit)	7.33
T_9(8.0mg/lit)	8.67
T_{10} (9.0mg/lt)	8.33
C.D.(0.01)	1.529

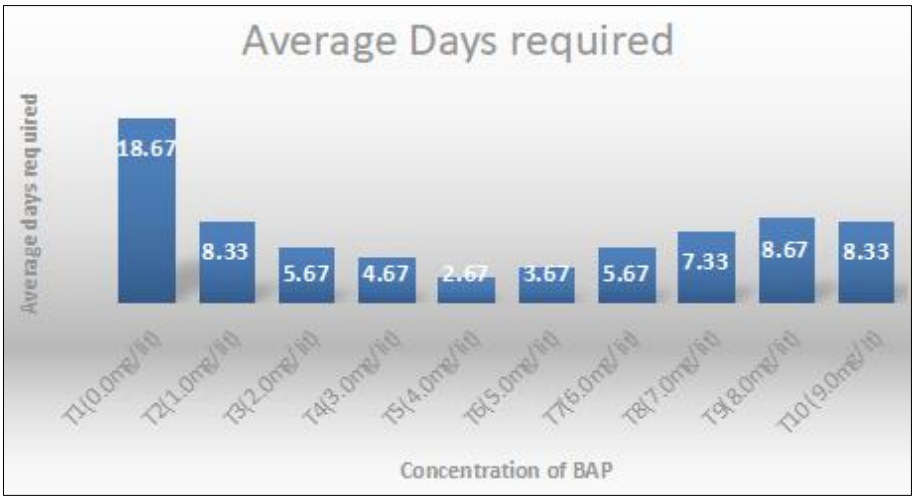

Fig 3: average days requiedfor shoot initiation

Data presented in table 4 and figure 3 clearly indicates that the average days required for shoot initiation is significantly influenced by different concentration of BAP. Treatment T5 (4mg/lt BAP) is found significantly superior over rest others but is at par with T6. Treatment T6 is at par with T4 and superior over rest others. Hence it is concluded that the BAP concatenation reduces days required for initiation of shoot.

3.5 Average number of leaves per explant

Data of average number of leaves per explant as influenced by different concentration of growth hormone BAP is given in table below

Table 5: Average number of days required for shoot initiation

Treatment (Concentration of BAP)	Average number of leaves
T_1(0.0mg/lit)	0
T_2(1.0mg/lit)	2.67
T_3(2.0mg/lit)	4.00
T_4(3.0mg/lit)	4.67
T_5(4.0mg/lit)	6.67
T_6(5.0mg/lit)	5.00
T_7(6.0mg/lit)	2.67
T_8(7.0mg/lit)	2.33
T_9(8.0mg/lit)	2.67
T_{10} (9.0mg/lt)	1.67
C.D.(0.01)	1.121

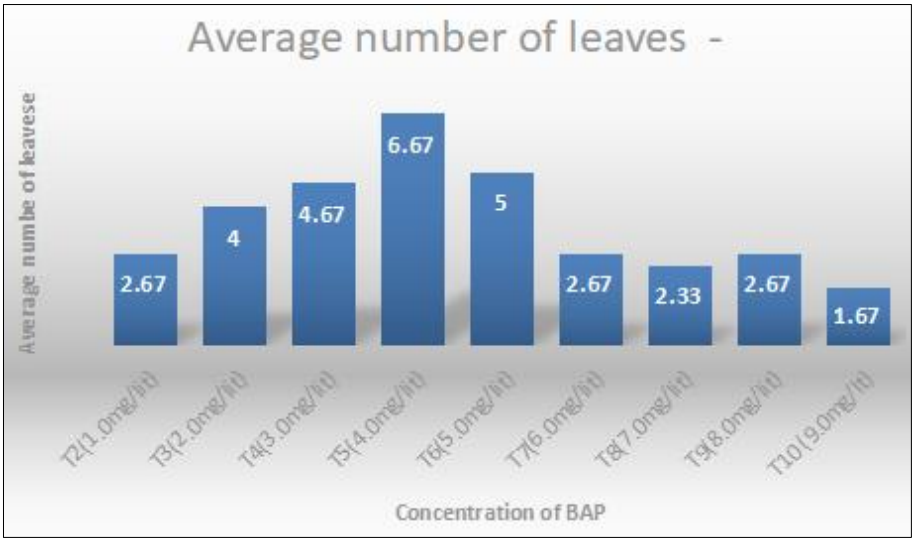

Fig 4: Average number of leaves per explant

Data presented in table 5 and figure 4 clearly indicates that the average number of leaves per explant is significantly influenced by different concentration of BAP. Treatment T5 is found significantly superior over rest other treatment for induction of leaves. Treatment T4 and T6 is at par with each other and superior over rest others. Hence it is concluded that the BAP concentration influences that number of leaves per explant.

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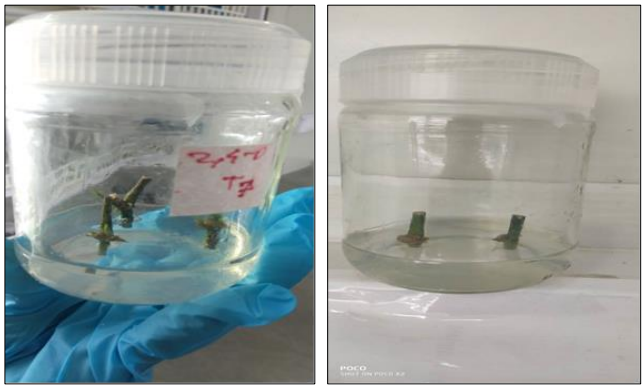

Plate 1: Inoculated Culture Bottle

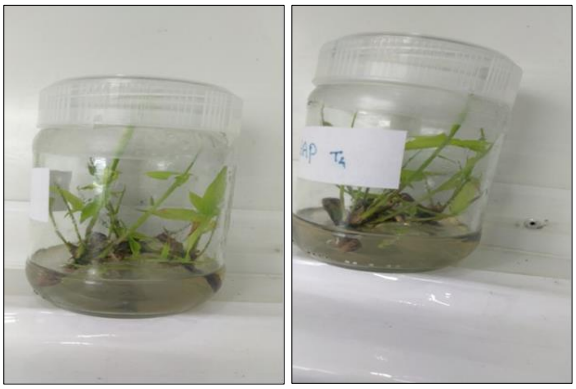

Plate 2: Multiple Shoot initiation and formation of leaves

References

1. Alexander, Rao. *In-vitro* culture of bamboo, Current status 1968;37:415.
2. Anonymous. Bamboo Forest News for Asia and the Pacific, FAO, Bangkok 1978; 2, 4.
3. Arya ID, Arya S. propagation of bamboos through tissue culture technology and field plantation. World Bamboo Congress, Thailand 2009, 131-143.
4. Arya Sharma S *et al*. Micropropagagation of *Dendrocalamus asper* by shoot proliferation, plant physiol 1962, 1999;15:473-497.
5. Austin AT, Marchesini VA. Gregarious flowering and death of understory bamboo slow litter decomposition and nitrogen turnover in a southern temperate forest in Patagonia, Argentina. Functional Ecology 2012; 26: 265–273.

6. Bag N, Chandra S, Palni LS, Nandi SK. Micropropagagation of Dev -ringal a temperate bamboo, and comparison between *in-vitro* propagated plants and seedlings. Plant science 2000;156:125-135.
7. Bereket H. Study on establishment of bamboo processing plants in Amhara Regional State. M.Sc. Thesis 2008;mAddis Ababa University, Addis Ababa, Ethiopia
8. Bhatt, David, Nirmala *et al*. Changes in nutrient components during emergency bamboo shoots, journals of food science 2005, 2007;(58) 612-618.
9. Chang, Ho. Multiple shoot induction and plant regeneration with uses, plant cell Rep 1997;18:691-695.
10. Das M, Pal A. *In-vitro* regeneration of bamboo, factors affecting changes of morphogenetic competence in axillary buds. Plant cell tissue organ culture 2005;81:109-112.
11. Geilis, Oprins, Dhooghe. Bambusa balcoa as basic for mass production of elite forestry bamboos. Plant cell tissue organ culture 1998;91:115-123.
12. Jimenez VM, Guevara E, Castillo J. *In-vitro* propagation of the neotropical giant bamboo through axillary shoot proliferation. Plant cell tissue organ culture 2006;86:389-395.
13. Kamble SY, Patil SR, Sawant S, Pawar. Studies on plant used in traditional medicine by Bhilla tribe of Maharashtra. Indian journal of traditional knowledge 2010;9(3):591-8.
14. Kassahun E. Ecological aspects and resource management of bamboo forests in Ethiopia.2003; Doctoral thesis submitted to Swedish University of Agricultural Sciences, Uppsala.
15. Kavita BM, Kiran S, An efficient technique for in- vitro propagation of Dendrocalamus Brandisii Kurz using nodal segment 2014;23:45-49.
16. Manpreet Sandhu, Shabir H, Wani. Victor M. Jimenez. *In-vitro* propagation of bamboo species through axillary shoot proliferation, plant cell tissue organ 2018;132:27-53.
17. Mehra SP, Mehra LK. Bamboo Cultivation- Potential and Prospects. Technical Digest. NABARD 2007; 10: 26-29.
18. Mehta U, Ramanuja Rao IV, Mohan RHY. Japanese Association of plant tissue culture, Tokyo 1982, 109-110.
19. Mudoi KD, Saikia SP, Goswami A, Gogoi A, Bora D, Borthakur M. Micropropagation of important bamboos: A review. African Journal of Biotechnology2013; 12 (20): 2770-2785.
20. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiologia plantarum 1962;15:473-497.
21. Nadgir AL, Phadke CH, Gupta PK, Mascarenhas AF. Rapid multiplication of bamboo by tissue culture. Silvae Gene 1984;33:219-223.
22. Pandey BN, Singh NB. Micro-propagation of *Dendrocalamus Strictus nees* from mature nodal explants. Journal of Applied and Natural Science 2012;4(1):5-9.
23. Panse VG, Sukhatme PV. Statistical Analysis for Agricultural Workers. 1967; ICAR, New Delhi.
24. Ravikumar R, Ananthakrishnan G, Ganapathi. *In-vitro* shoot propagation of Dendrocalamus Strictus Need. Plant cell tissue organ culture 1998;52:189-192.
25. Reddy GM. Clonal propagation of bamboo Dendrocalamus Strictus. Current science 2006;11:1462-1464.
26. Saxena S, Dhawan V. Regeneration and large scale propagation of bamboo Dendrocalamus Strictus, plant cell Rep 1999;18:438-3.
27. Singh M, Jaiswal U. Induced in- vitro flowering in flowering in Dendrocalamus Strictus Need. Current science 2010;79:1529-1530.
28. Singh SR, Singh R, Kalia S, Dalal S, Dhawan AK, Kalia RK. Limitations, progress andprospects of application of biotechnological tools in improvement of bamboo-a plant with extraordinary qualities. Physiology and Molecular Biology of Plants 2013; 9 (1): 21-41.
29. Sood A, Ahuja PS, Sharma OP, Godbole S. *In-vitro* protocol and field performance of elites of an important bamboo Dendrocalamus hamiltonii Need et Arn. Ex. Munro. Plant cell tissue organ culture 2002;71:55-63.
30. Subramanlam KN. Bamboo demand and supply of planting stock. INBAR news lett 1994;5:24-25.
31. Varmah JC, Pant MM. The Production and Utilization of Bamboos. The Indian Forester 1981; 107: 465-476.
32. Vongvijitra R. Traditional vegetative propagation and tissue culture of some Thai bamboos, Current research India 1998, 159-166.
33. Yuan JL, Gu XP, Li LB, Yue JJ, Yao N, et al. Callus induction and plantlet regeneration of Bambusa multiplex. Scientia Silvae Sinica 2009; 45:38–42 (in Chinese).
34. Zamora AB. Review of micropropagation research on bamboos. INBAR Technical Report No. 5, New Delhi, International Network for Bamboo and Rattan 1994, 45-100.