Biological Forum – An International Journal **13(3a): 221-227(2021)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Development of Sterilization and Initiation Protocol for *in vitro* Regeneration in Bamboo (*Bambusa balcooa* Roxb.)

Ananthu J.S.[1*], S.V. Pawar[2], B.R. Najan[3], Y.M. Raut[4] and Vaibhav Parmeshwar Gulwane[1]

[1] M.Sc. Scholar, Department of Agricultural Botany,
Post Graduate Institute, MPKV, Rahuri, (Maharashtra), India.
[2]Associate Professor, Department of Agricultural Botany,
College of Agriculture, Pune (Maharashtra), India.
[3]Associate Professor, Department of Agricultural Botany, AICRP on Agroforestry,
MPKV, Rahuri, (Maharashtra), India.
[4]Senior Research Fellow, Bamboo Plantation Project,
State Level Biotechnology Centre, MPKV, Rahuri, (Maharashtra), India.

(Corresponding author: Ananthu J.S.)*
(Received 01 July 2021, Accepted 09 September, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: In bamboo, propagation is usually done by two main methods. First by propagation of bamboos by using offsets, culm or side branch cuttings and second way is through micropropagation, also called as clonal propagation, which is best achieved through tissue culture techniques. *In vitro* propagation is frequently used for meeting the ever-growing demand for this bamboo species due to its multipurpose nature. The present investigation on development of sterilization and initiation protocol for *in vitro* regeneration in bamboo (*Bambusa balcooa* Roxb.) was undertaken to identify suitable explants and media for its micropropagation. The experiment was conducted using nodal explants collected from Bamboo Plantation Project, MPKV, Rahuri and the current research was conducted in State Level Biotechnology Centre, MPKV, Rahuri. Nodal explants as explants and MS media as basal media were found ideal for *in vitro* culture. In case of sterilisation of nodal explants, sterilisation done with Tween 20 (10 min) + 1% Bavistin (7min) + 0.1% $HgCl_2$ (5min) + 70% ethanol with 3 times washing with distilled water in between each sterilants, showed the least contamination percentage of 9.5%. Different hormones were experimented out in MS media with nodal explants to study their response in shoot initiation. In case of shoot initiation BAP (1mg/l) showed top results in MS media where 75% of explants have shown initiation as early as 7 days after inoculation, whereas when BAP (1mg/l) is mixed with TDZ (1mg/l), maximum number of shoot initiation occurred.

Keywords: Nodal explants, Sterilization, Initiation, MS media, Standard error.

INTRODUCTION

Bambusa balcooa Roxb., belongs to subfamily Bambusoideae, tribe Bambuseae and is an important multipurpose species in India and surrounding regions. *B. balcooa* typically occurs at altitudes of up to 600m, with a preference towards soil conditions having good drainage and heavy texture. *B. balcooa* has multiple uses and it is because of this multipurpose nature of the bamboo, that this species of bamboo has been identified by the National Mission on Bamboo (NBM) India as one of the species for large scale cultivation. As per Grand View Analysis, a recent study was done which suggested that by 2025, the international market potential of bamboo is predicted to cross USD 98.3 billion (Grand View Research, 2020). It is an extremely sturdy bamboo and is a preferred bamboo species for scaffolding. It is also used as raw material for paper making by the paper and pulp industry and for making agarbatti sticks along with *Bambusa tulda* by the agarbatti industry. Because of the multipurpose usefulness of bamboo, it is being encouraged for production in several places in world (Takouleu, 2020; National Bamboo Mission, 2020).

Bambusa balcooa has its origin in north-eastern part of India from where it has spread to other locations due to its multiple uses. This species was known to be introduced in the villages of Terai region of Uttarakhand by fleeing refugees from southern Bengal during the time of partition. Now this species can be seen right across India and even up to the southern tip of Kerala (Banik, 2000).

Bamboo has been a part of the traditional cuisine of the South Asian countries, along with India, in several food preparations (Singhal *et al.*, 2013; Sarkar *et al.*, 2020). It is highly appreciated for its solid culms; and it is considered as one of the distinguished species for building and scaffolding purposes (Tewari, 1992; Minke, 2012; Banik, 2016). Owing to the ban on plastics, the prevalence of bamboo bottles has increased in many places, in which *B. balcooa* being used (Sreejith, 2020). Due to its rapid growth, yield potential, high survival rate and multipurpose uses, it has been considered as one of the most prominent bamboo species with industrial significance (Tewari *et al.*, 2014; Yeasmin *et al.*, 2015; Krishnakumar *et al.*, 2017). The demand for good quality *B. balcooa* have been increasing a lot in the last few years due to the multipurpose uses,

which has inspired many farmers to cultivate bamboo (Fernandes, 2017).

Traditionally, the plant is propagated via vegetative methods, especially during summer (Pattanaik *et al.*, 2004; Ray and Ali, 2017). However, it is not possible to compensate the huge demand of farmers using the vegetative methods. Also, the chances of soil born disease transmission are very high. Whereas, micropropagation technique offers large-scale propagation of healthy and genetically uniform plants irrespective of the season. Plant tissue culture techniques have certain advantages over traditional propagation methods.

Although, earlier attempts were made for micropropagation of *Bambusa balcooa* Roxb (Das and Pal, 2005; Gillis *et al.*, 2007; Mudoi and Borthakur, 2012; Rathore *et al.*, 2009; Negi and Saxena, 2011; Sharma and Sarma, 2011; Choudhary *et al.*, 2016; Chandra *et al.*, 2018), however there are still considerable efforts to be made for making it more practicable. One of the largest biotech companies dealing in bamboo in India is Grow more Biotech India Ltd., Hosur. This company has produced 6 million plantlets of *B. balcooa* annually through tissue and branded it as 'Beema' bamboo, in a reference to its extraordinary strength properties after the mythical character.

MATERIALS AND METHODS

All the chemicals used were of analytical grade in the current study. Experiments were carried out for finding efficient growth of explants in Woody Plant Media and Murashige and Skoog Media (Murashige, & Skoog 1962). Once the MS media showed favourable results for *in vitro* regeneration in *Bambusa balcooa*, it was utilized throughout the course of research. Once the stock solutions of different components were prepared, the media was formulated by combining these stock solutions in such a manner so as to balance the final amount of each. Stock solution for the plant growth hormones were made by combining desired concentration of auxins and cytokinins. The auxins (IAA and NAA) were initially dissolved in some drops of absolute alcohol whereas, cytokinins (BAP and Kn) were dissolved in 10 ml of 1N NaOH and thereafter the final volumes were bought up by adding required amount of double distilled water for upto one litre. Storage of stock solutions of various growth regulators done in a refrigerator and used between 2 weeks of its preparation. For all purposes of the work, double distilled water was always used. The pH of medium was fixed at 5.8 and adjusted accordingly using 1N NaOH or 1N HCl solution before it was autoclaved. Before autoclaving, plant growth hormones were added to the basal medium. Media used for present experiment was Murashige and Skoog (MS) media as a basal medium for initiation. For all the stages of experiment, borosilicate glasswares were taken. Glass wares were initially washed with Tween 20 biodegradable detergent and thereafter washed properly with tap water for removing the detergent completely. Finally, they were rinsed with distilled water and sterilization is done in oven at a temperature of 160-180°C for about 3-4 hours.

Autoclaving: Sterilization of media was done in autoclave. Micronutrient combinations, distilled water, and other stable mixtures prepared were autoclaved. Autoclaving the culture media contained in glass containers were carried out at 15 psi. pressure and 121°C temperature for about 15- 40 minutes. Exposure time for autoclaving is dependent on volume of liquid which is needed to be sterilized. Maximum care was taken during the procedure. After the media is autoclaved, it was stored in dark environment for 48 hours at 25±2°C.

Culture room sterilization and transfer area: Initially, cleaning of culture rooms was done by gently washing the floors and walls by using a detergent soap and then cleansing with phenyls daily. Sterilization of transfer area was done by exposing it to UV light. Installation of HEPA filter ventilation unit checked that aseptic condition of transfer area is maintained. Sterilisation of laminar airflow hoods were done by wiping the working surface using 70% ethyl alcohol.

Explants preparation and sterilization: Obtaining sterilized explants is complicated because during the sterilization process biological activity of living material should be maintained and only the fungal and bacterial contaminant needs to be eliminated. Various surface sterilization agents were used for sterilisation of explants. Nodal segment with 2-3 nodes were taken and used as explants for studying the effect of sterilisation. Further trimming of the collected explants to desired sizes with sterile scalpel blade was done. Once the explants were cut to suitable size having a single node, they were washed well in the running tap water for about 20 minutes and then they were treated with or without different sterilants (Washing 3 times with sterile distilled water between each step) for studying their effects on sterilisation and treatment numbers were given from T1 to T6.

Inoculation of explants: Inoculation of the explants on culture media aseptically was done after sterilisation process. For doing inoculation, the explants were shifted to large sterilized glass petri plates by using sterile forceps done under strict aseptic conditions. These explants were then inoculated vertically to culture bottles which contain MS medium added with different plant growth hormones. Once the explants are vertically inoculated in culture by keeping the node above medium, the culture bottle's mouth was quickly flamed and closed with cap.

Culture conditions: All the cultures were incubated at a temperature of 25±2°C under fluorescent light in 16: 8 hour's photoperiod and light intensity of 2500 lux.

Effect of media and type of explant used: Various media like Murashige and Skoog Medium and Woody Plant Medium were tested for direct shoot formation from nodal segment and shoot apex explants at most responsive level of different plant growth regulators.

Effect of plant growth hormones on shoot initiation: Three types of hormone combinations were incorporated in media. Each type is done in different hormone concentrations as follows:

(a) **BAP added singly in 1 L medium.** BAP (0.5mg/l, 1mg/l, 2mg/l, 3mg/l, 4mg/l, 5mg/l)

(b) **BAP and TDZ added in combination in 1 L medium.** BAP (1mg/l, 2mg/l) + TDZ (1mg/l, 2mg/l, 3mg/l)

(c) **BAP and kinetin added in combination in 1L medium.** BAP (1mg/l, 2mg/l) + kinetin (1mg/l, 2mg/l, 3mg/l)

After around 3 weeks from shifting explants to inoculation media, number of days required for shoot initiation and sum total of shoots formed for explants were recorded.

Observations recorded: Each treatment was replicated 3 times and then the whole experiment was repeated twice again for getting unbiased results. Observation of cultures were done periodically and following recording of observations were done:

I. Effect of treatment of nodal explants with various sterilants.

II. Shoot initiation percentage.

III. Number of shoots/explant.

Completely randomized design was used for conducting the experiment. Data for initiation of explants collected were analysed for mean and standard error as given by Snedecor and Cochran (1972). Calculation of standard error for number of shoot formation was done after their respective value transformation of shoot induction was made from the explants. Each value of replication was then transformed by using square root transformation method as follows:

$$\sqrt{Y + 1/2}$$

Where, Y= original value of observation

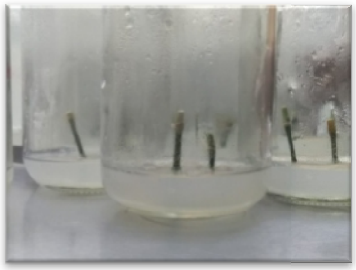
Year of experimentation: 2019-2021

Place of experimentation: State Level Biotechnology Centre, PGI, MPKV, Rahuri-413 722 Ahmednagar Distt., Maharashtra, India.

RESULT AND DISCUSSION

In the current study conducted, out of the two types of explants taken (shoot apex and nodal segment), only nodal explants have showed positive results with regards to initiation, while in case of shoot apex explants, the explants dried off. The results are also in par with experiments done by Nieri *et al.,* (2020) on *D. giganteus* where they found out that binodal propagules proved to be efficient for their propagation. When two different media were tested for shoot initiation using nodal explants i.e, in Murashige and Skoog Media and Woody Plant Media, MS media supplemented with 1mg/l BAP showed better and healthy initiation of shoots in explants, whereas the results observed on Woody Plant Media were poor, since the explants dried up without showing initiation. Thus, nodal explants in MS media were used throughout the research. Out of the six treatments used for sterilization, treatment number T6 showed the least contamination percent of 9.5, whereas T3 showed the highest contamination % of 36.3 as shown in Table 1. Surface sterilized explants and inoculation of explants are shown in Plate 1.

Surface sterilized explants in glass bottle.



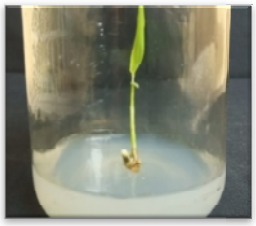
Inoculation of sterilized explants on solid MS medium.

Plate 1: Surface sterilized explants and inoculation of explants.

Table 1: Effect of treatment of nodal explants with various sterilants.

Treatment code	Sterilisation of explants	% of explants that showed initiation without any contamination (x)	Contamination percentage(y), where, y= 100-x
T1	Tween 20 (5 min) + 1% Bavistin (7 min) + 0.1% $HgCl_2$ (5 min)	70.5	29.5
T2	Tween 20 (10 min) +1% Bavistin (10 min) + 0.1% $HgCl_2$ (5min)	72.4	27.6
T3	Chlorex 3% (5 min) + 1% Bavistin (7 min) + 0.1% $HgCl_2$ (5min)	63.7	36.3
T4	Chlorex 3% (10 min) + 1% Bavistin (10 min) + 0.1% $HgCl_2$ (5min)	68.6	31.4
T5	Tween 20 (5 min) + 1% Bavistin (10 min) + 0.1% $HgCl_2$ (5min) + 70% ethanol	83.5	16.5
T6	Tween 20 (10 min) + 1% Bavistin (7 min) + 0.1% $HgCl_2$ (5min) + 70% ethanol	90.5	9.5

Three types of hormone combinations were incorporated in media (BAP applied singly, BAP + TDZ, BAP + kinetin) for initiation and the nodal explants were inoculated on it after sterilisation. In case of BAP supplemented alone in MS Media, the nodal explants showed shoot initiation between 7 to 10 days of inoculation at different levels of BAP. Treatment names were given as BI 1-5 (Basal initiation media 1-5). Highest number of shoot bud initiation was recorded at 1mg/l BAP (2.83) at a success rate of 75% on treatment BI2, whereas the lowest number of shoot buds (1.91) were observed at 5mg/l BAP with 41 % success rate on treatment BI6. Here, increasing concentration of BAP above 1mg/l showed a reduction in the shoot bud initiation progressively. The results obtained are shown in Table 2 and Plate 2 shows initiation of nodal explants on solid medium containing BAP. Mudoi and Borathakur, (2012) also found that 1.0 mg/l BAP was better for shoot initiation. They reported that axillary bud growth was found after 8 days of inoculation with better percentage of shoot initiation. The present results are also in agreement with the results obtained by Kapruwan *et al.,* (2018) where they obtained maximum shoot initiation in MS medium containing BAP.



Two weeks after inoculation.

Four weeks after inoculation.

Plate 2: Initiation of nodal explants on solid medium containing BAP.

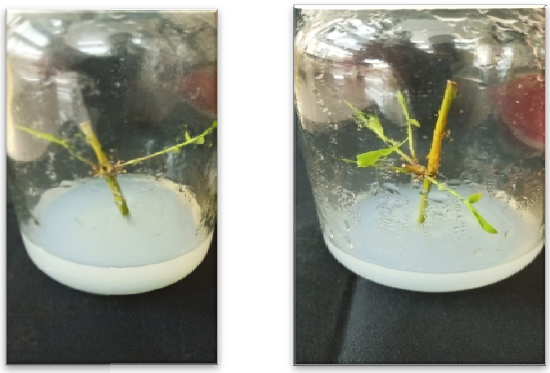
Table 2: Effect of BAP on shoot initiation of *Bambusa balcooa* nodal explants on MS medium.

Treatment name	Concentration of BAP (mg/l)	% of shoot initiation	Number of days taken for shoot initiation (Mean ± SE)	Number of shoots/ explant (Mean ± SE)
BI 1	0.5	60	8±1.30	2.2±0.19
BI 2	**1**	**75**	**7±1.22**	**2.83±0.13**
BI 3	2	58	8±1.26	2.42±0.17
BI 4	3	55	10±1.29	2.16±0.15
BI 5	4	49	9±1.21	1.73±0.11
BI 6	5	41	10±1.25	1.91±0.16

In case of BAP + TDZ taken together, the nodal explants which were inoculated on media shown initiation of shoots between 8 to 12 days of inoculation. Treatment names were given as BI 7-12 (Basal Initiation Media 7-12). Highest number of shoot bud initiation was observed at 1mg/l BAP +1 mg/l TDZ (3.15) at a success rate of 63% in BI7 treatment, whereas the lowest number of shoot buds (1.98) were observed at 2mg/l BAP + 3mg/l TDZ with 40 % success rate on BI12 treatment. Here, increasing concentration of BAP and TDZ above 1mg/l showed a reduction in the shoot bud induction progressively. The results obtained are shown in Table 3 and Plate 3 shows initiation of nodal explants on solid medium containing BAP and TDZ. The results obtained for sum total of shoots are in par with the results recorded by Gusmiaty *et al*., (2020). They also studied on MS medium and recorded that TDZ concentrations significantly affected by the number of shoots. The addition of thidiazuron (TDZ) into media resulted in the best percentage of shoot number (80%) in all concentrations of media in their experiment.

In case of last combination for shoot initiation used, i.e., BAP + Kinetin, the nodal explants which were inoculated on media shown initiation of shoot between 9 to 12 days of inoculation at different combinations of BAP and kinetin. Treatment names were given as BI 13-18 (Basal Initiation Media 13-18). Highest number of shoot bud initiation was observed at 1mg/l BAP + 2 mg/l Kinetin (2.62) at a success rate of 60% on treatment BI14, whereas the lowest number of shoot buds (1.08) were observed at 2mg/l BAP + 3mg/l Kinetin with 43 % success rate on BI18 treatment. Here, increasing the concentration of BAP above 1mg/l and Kinetin above 2mg/l showed a reduction in shoot bud induction progressively. The results obtained here are in par with the experiments done by Azin and Forogh (2015) on micropropagation of rose, where they used Kinetin along with BAP and obtained best shoot growth on MS media. Negi and Saxena (2018) also used BAP + Kinetin hormone combinations in *Bambusa nutan*. They used *Bambusa nutan* for large scale multiplication using MS medium which was supplemented with benzyl aminopurine (BAP) and

Kinetin (Kn) gelled with 0.2% gelrite through which the yields received was 80% aseptic culture in shoot initiation with appreciable result. The results obtained are shown in Table 4 and Plate 4 shows initiation of nodal explants on solid medium containing BAP and Kinetin.



Two weeks after inoculation. Four weeks after inoculation

Plate 3: Initiation of nodal explants on solid medium containing BAP and TDZ.

Table 3: Effect of BAP and TDZ in combination on shoot initiation of *Bambusa balcooa* nodal explants on MS medium.

Treatment name	Concentration of BAP + TDZ (mg/l)	% of shoot initiation	Number of days taken for shoot initiation (Mean ± SE)	Number of shoots/ explant (Mean ± SE)
BI 7	**1+1**	**63**	**8±1.30**	**3.15±0.19**
BI 8	1+2	61	9±1.33	2.8±0.13
BI 9	1+3	55	9±1.29	2.75±0.17
BI 10	2+1	54	10±1.21	2.56±0.15
BI 11	2+2	45	12±1.23	2.16±0.11
BI 12	2+3	40	11±1.28	1.98±0.16

Two weeks after inoculation. Four weeks after inoculation.

Plate 4: Initiation of nodal explants on solid medium containing BAP and Kinetin.

Table 4: Effect of BAP and Kinetin added in combination for shoot initiation of *Bambusa balcooa* nodal explants on MS medium.

Treatment name	Concentration of BAP + Kinetin (mg/l)	% of shoot initiation	Number of days taken for shoot initiation (Mean ± SE)	Number of shoots/ explant (Mean ± SE)
BI 13	1+1	58	10±1.31	2.44±0.11
BI 14	**1+2**	**60**	**9±1.29**	**2.62±0.18**
BI 15	1+3	56	9±1.27	2.39±0.19
BI 16	2+1	52	10±1.24	2.28±0.25
BI 17	2+2	45	12±1.28	1.86±0.19
BI 18	2+3	43	11±1.20	1.08±0.17

SUMMARY AND CONCLUSION

Among different bamboos, *Bambusa balcooa* is one among the most important and desired species of farmer for raising plantation mainly due to its multipurpose utility. Since unknown past, the species has been extensively cultivated and became a very popular bamboo for the farmers. It grows well in tropical humid conditions. It also has a high Calorific value and Fuel Value Indices (FVI) which makes it suitable for Bio- energy plantations and gasification. *Bambusa balcooa* is suitable for paper and pulp industry. It shows superior strength properties including good culm wall thickness. Life cycle of

clump is more than 50 years and also has an added advantage as it shows minimal pest problems and no gregarious flowering in India. Due to the increasing demand of *Bambusa balcooa*, *in vitro* regeneration of this bamboo has gained importance as it enables mass multiplication in a small period of time and thus it offsets the limitations of traditional propagation methods followed earlier.

An efficient protocol for sterilization and initiation of *Bambusa balcooa in vitro* was found out in the present research. Among the two media used, Murashige and Skoog Media showed shoot initiation compared to Woody Plant Medium, where the explants dried off and thus MS media was used for further studies. Nodal explants and shoot apex explants were used to find out their efficiency in regeneration. Nodal explants showed shoot initiation, whereas shoot apex explants dried off. Thus, different hormones were tried out in MS media with nodal explants to study their response in shoot initiation.

In case of sterilisation of nodal explants, sterilisation done with Tween 20 (10 min) + 1% Bavistin (7min) + 0.1% $HgCl_2$ (5 min) + 70% ethanol with 3 times washing with distilled water in between each sterilants, showed the least contamination percentage of 9.5% compared to other treatments experimented and subsequently this procedure was adopted throughout the research for sterilisation of explants which is taken for initiation.

In case of shoot initiation, BAP (1mg/l) showed best results in MS medium where 7% of explants showed initiation as early as 7 days after inoculation, whereas when BAP (1mg/l) is mixed with TDZ (1mg/l), maximum number of shoot initiation occurred, with a mean of 3.15 shoots.

Thus, using the above protocol for sterilization and initiation of *Bambusa balcooa* Roxb., it increases the efficiency of obtaining a larger amount of *in vitro* grown plantlets at a faster rate and helps in meeting the ever -growing demand for several multipurpose bamboo species.

Acknowledgement. We sincerely thank State Level Biotechnology Centre, MPKV for providing all the needful resources to carry out the research and also for PGI, MPKV, Rahuri for providing a platform for conducting this project.

Conflict of Interest. The author declares no conflict of interest.

REFERENCES

Azin, H., & Forogh, M. (2015). Effect of Spermidin and Cytokinin on in vitro Induction Flowering & Micropropagation of Rosa sp. (*Dolsevita*). *Biological Forum – An International Journal*, *7*(2): 56-62.

Banik, R. L. (2000). Silviculture and field-guide to priority bamboos of Bangladesh and South Asia. Published by Bangladesh Forest Research Institute, Chittagong.

Banik, R. L. (2016). *Bambusa*. Silviculture of South Asian Priority Bamboos, *Tropical Forestry Journal.* Springer Singapore, Singapore, pp. 21–115.

Chandra, K., Sahu, K., Kaladhar, D. S. & Belorkar, S. A. (2018). A rapid *in vitro* propagation of *Bambusa balcooa* by plant tissue culture technique. *African journal of Biotechnology*, *8*: 27-31.

Choudhary, A. K., Ranjan, A., & Kumari, P. (2016). *In vitro* shoot proliferation for rapid and mass production of quality planting materials of bamboo species *Bambusa nutans* in the climatic conditions of Bihar, India. *Indian Journal of Energy*, *5*(2): 1-11.

Das, M., & Pal, A. (2005). *In vitro* regeneration of *Bambusa balcooa* Roxb.: Factors affecting changes of morphogenetic competence in axillary buds. *Plant Cell, Tissue and Organ Culture*, *81*: 109–112.

Fernandes, V. (2017). Bamboo Can Be More Profitable Than Sugarcane and Rice!. Financial Express.

Gillis, K., Gielis, J., Peeters, H., Dhooghe, E., & Oprins, J. (2007). Somatic embryogenesis of mature *Bambusa balcooa* Roxburgh as a basis for the mass production of elite forestry bamboos. *Plant Cell, Tissue and Organ Culture*, *91*: 115–123.

Grand View Research, (2020). Bamboos Market Size is worth $98.3 Billion By 2025 | CAGR: 5.0% [Website]. URL *https://www.grandviewresearch.com/press-release/global-bamboos-market*

Gusmiaty, W., Hiswan, J., Larekeng, S. H., & Putri, S.D. (2020). *In vitro* shoot induction of Batik Bamboo using BAP and NAA. *Plant Archives*, *20*(2): 2343-2349

Kapruwan, S., Bakshi, M., & Kaur, M. (2018). Rapid *in vitro* propagation of solid bamboo, *Dendrocalamus strictus* Nees, through axillary shoot proliferation. *Bamboo International*, *7*(3): 56-68.

Krishnakumar, N., Kanna, S. U., Parthiban, K. T., & Rajendran, P. (2017). Physical properties of the thorn less bamboos (*Bambusa balcooa* and *Bambusa vulgaris*). *Agricultural Update*, *12*: 15740.

Minke, G. (2012). Building with Bamboo. Birkh¨user GmbH, Basel Birkh¨user. Basel P.O. Box, CH-4002 Basel, Switzerland Part of De Gruyter.

Mudoi, K. D., & Borthakur, M. (2012). Factors which affect the frequency of *in vitro* flowering of *Bambusa balcooa* Roxb. *Indian Journal ofPlant Physiology*, *17*: 37–43.

Murashige, T., & Skoog, F. (1962). A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Plant Physiology*, *15*: 473-497.

National Bamboo Mission (2020). National Bamboo Mission, Ministry of Agriculture & Farmers Welfare Government of India [Website]. URL *https://www.nbm.nic.in*

Negi, D., & Saxena, S. (2018). Micropropagation of *Bambusa balcooa* Roxb. through axillary shoot proliferation. *In vitro Cell Developmental Biology-Plant*, *47*: 604–610.

Nieri, E. M., de Almeida, R. S., & Cartolano, R. T. (2020). Methods of preparing propagules for tropical woody bamboo species. *Scientia Agraria Paranaensis*, *19*(2): 196-199

Pattanaik, S., Das, P., Borah, E., Kaur, H., & Borah, K. (2004). Vegetative multiplication of *Bambusa balcooa* Roxb. by using branch cuttings. *Journal of Bamboo and Rattan*, *3*: 365–374.

Rathore, T. S., Kabade, U., Jagadish, M. R., Somashekar, P. V., & Viswanath, S. (2009). Micropropagation and evaluation of the growth performance of selected industrially important bamboo species

from southern India. World Bamboo Congress Proceedings, 16-19 November 2009. Bankok, Thailand, pp. 41–55.

Ray, S. S., & Ali, M. N. (2017). Factors affecting macropropagation of bamboo with special reference to culm cuttings: a review update. *New Zealand Journal of Forestry Science*, *47*: 17.

Sarkar, D., Chandra, A. K., Chakraborty, A., Ghosh, S., Chattopadhyay, S., Singh, L. H., & Ray, I. (2020). Effects of bamboo shoots (*Bambusa balcooa*) on thyroid hormone synthesizing regulatory elements at cellular and molecular levels in thyrocytes. *Journal of Ethnopharmacology, 250*: 112463

Sharma, P., & Sharma, K. P. (2011). *In vitro* propagation of *Bambusa balcooa* for a better environment. International Conference on Advances in Biotechnology and Pharmaceutical Sciences pp 248–252.

Singhal, P., Bal, L.M., Satya, S., Sudhakar, P. & Naik, S. N. (2013). Bamboo shoots: a novel source of nutrition and medicine. Crit. Rev. *Food Science and Nutrition*, *53*: 517–534.

Snedecor, G. W., & W. G. Cochran, (1972). Statistical Methods. 6th ed. Iowa University Press, pp.593.

Sreejith, T. J. (2020). Bamboo Bottles Instead of Plastic: Kerala to Follow Sikkim Trend [WWW Document]. Mathrubhumi (English Ed.) URL *https://english.mathrubhumi. com/news/good-news/bamboo-bottles-instead-of-plastic-kerala-to-follow-sikkim-trend-1.4592078*

Takouleu, J. M. (2020). CONGO: € 152 Million Project for the Promotion of Bamboo Products [Website]. AfriK21. URL *https://www.afrik21.africa/en/congo-e152-million-project-for-the promotion-of-bamboo-products*

Tewari, D. N. (1992). *A Monograph on Bamboo*. International book distributors, Dehradun, India, pp. 31–32.

Tewari, S., Kaushal, R., Tewari, L., & Chaturvedi, S. (2014). Evaluation of bamboo species in India: results from a multilocation trial. *Indian Journal of Agroforestry*, *16*: 68–73.

Yeasmin, L., Ali, M. N., Gantait, S., & Chakraborty, S. (2015). Bamboo: an overview on its genetic diversity and characterization. *Biotechnology*, *5*: 1–11.

How to cite this article: Ananthu J.S., Pawar, S.V., Najan, B.R., Raut, Y.M. and Gulwane, V.P. (2021). Development of Sterilization and Initiation Protocol for *in vitro* Regeneration in Bamboo (*Bambusa balcooa* Roxb.). *Biological Forum – An International Journal*, *13*(3a): 221-227.