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In vitro regeneration of bamboos, the "Green Gold": An overview

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Bamboo is a versatile, non-timber, forest product having glorious history. Being a vital resource, there is an ever increasing demand for this 'Green Gold', but conventional breeding is severely handicapped because of two main reasons, *i.e.*, long vegetative phase and irregular flowering. Thus, to fulfill the requirement of demand, the best way is to switch to scientific techniques. *In vitro* culture offers an alternative option for producing desirable clones in stipulated period of time. Among the different explants used for micropropagation of bamboos, young branch node was the most preferred. Murashige and Skoog's medium supplemented with sucrose and plant growth regulators (PGRs) were extensively used in the regeneration of bamboos. PGRs like 6-benzylaminopurine (BAP), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), kinetin (Kn), 1-naphthaleneacetic acid (NAA), 2,4 dichlorophenoxyacetic acid (2,4-D) were mostly preferred over others for bamboo micropropagation, while 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and gibberellic acid (GA_3) were also used in some cases. Direct *in vitro* shooting was preferred in regeneration of bamboos over somatic embryogenesis through callus culture. *In vitro* rhizogenesis was well achieved in presence of phytohormones but was not solely depended on them. Further, regenerated plantlets were successfully established in the field with high rate of survival. In the present review we endeavor to give a consolidated account of *in vitro* regeneration of bamboos starting from the pioneer work initiated in 1968.

Keywords: Bamboo, micropropagation, plant growth regulators (PGRs), regeneration; rhizogenesis

Introduction

Bamboo has been associated with mankind since ages and has been an indispensable part of almost every civilization. Bamboo is a general term used for about 1575 species of tall arborescent grasses belonging to subfamily Bambusoideae of the grass family Poaceae[1,2]. There are over 1500 documented uses of bamboo worldwide[3,4]. Today bamboo is globally recognized as an important asset in eradication of poverty, economic and environmental development, and thus establishing its image as to "Green Gold"[5]. Throughout the globe, bamboo meets the basic requirements of the common people. Bamboo is closely associated with indigenous culture and knowledge not only in Asia but also in Africa and Latin America. Bamboo has multifarious uses and serves as a superior material for constructions, utensils, weapons, fuel, fodder, food, firewood, furniture, mats, chop sticks, tooth picks, handicrafts, musical instruments etc[6]. It is extensively used in the paper and pulp industries. With the development of science and technology, the uses of bamboo have also expanded. Today, bamboo is used in making hard boards, flooring, corrugated sheets etc.[7] and thus can serve as a replacement of wood. Apart from this, the shoots of bamboo are used as delicacy because of their high nutritive values. The shoots are rich in proteins, saccharides, amino acids and minerals, while low in fat; and the water content may be as high as 90%[8]. The shoots are used in many exquisite culinary preparations like pickle, vegetables, soup, salads, vinegar and several other forms in different countries[9].

The use of bamboo as traditional medicine by the Chinese dates back to over 1000 years[10]. Recently, many biologically active components in bamboo leaves and their potential health benefits have been widely studied[11-18]. Thus it can safely be asserted that each part of bamboo is not only a treasure but also a medicine. The use of bamboo is endless and because of this it is an indispensable resource for the rural people. In fact, bamboo is valuable from top to rhizomes. Bamboo, though being a vital resource, has not easily lent itself to modern methods of macropropagation and genetic improvement because of its long vegetative phase and monocarpic flowering behaviour[19]. The conventional breeding also seems to be difficult because of the erratic flowering. Thus,

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modern scientific methods must be applied to manage the bamboo forest. Tissue culture is playing a major role in realizing this objective for the bamboo production to meet the demand. Tissue culture offers enormous potential in producing large quantities of the desired material in a short time frame[20]. However, it is essential that enough care is taken in selection of the initial material, production of the plants, nursery development and field plantation. Bamboo plantation is by and large through seeds, offsets and culm cuttings[21]. The *in vitro* methods offer an attractive alternative to conventional methods for the mass propagation of bamboos[22]. Enhancement in research and development (R & D) activities for genetic improvement in bamboo, development of efficient methods for mass production of superior quality planting stock and conservation of the genetic resources is of absolute necessity.

Micropropagation—A Tool For Regeneration

According to Murashige[23], there are three possible methods available for micropropagation, *viz*., enhanced release of axillary buds, and production of advantageous shoots through organogenesis and somatic embryogenesis.

In case of shoot tip, nodal and axillary bud cultures, clonal fidelity is conserved to a greater extent. However, in case of callus mediated organogenesis and somatic embryogenesis, there is a risk of producing aberrants and thus is not recommended for clonal propagation. Though limited to a few species, *in vitro* somatic embryogenesis tends to be the most effective and rapid method of plant regeneration[24]. Currently, *in vitro* micropropagation has been adopted for a number of economically and medicinally important plant species and bamboo is no exception.

Importance of *In Vitro* Regeneration of Bamboos

In bamboo, breeding is seriously handicapped because of its long vegetative phase, monocarpic flowering behavior and poor seed set[19]. Moreover, it is near impossible that two desirable plants will flower simultaneously; therefore, conventional breeding also seems to be difficult. Thus for meeting the raw material demand, the best possible way to manage the bamboo forest is through scientific management. Major limitations to bamboo production can be overcome by propagation methods. *In vitro* culture offers a method for producing variations and exploring the resultant variations for crop improvement. *In vitro* culture techniques also provide an alternative means of plant propagation and a tool for crop improvement[25]. Further, *in vitro* regenerated plants are superior to conventionally propagated plants in respect of productivity and disease resistance.

Establishment of Culture and Regeneration

The main aim here is to successfully and aseptically transfer the explants into culture medium and then provide *in vitro* environment for growth and differentiation. The important aspects of this process are explant disinfection, explant selection and culture medium[26]. The success of establishment of culture *in vitro* depends on the selection of explant, sterilization of explant, composition of the culture media and finally on culture conditions provided for growth and development. The credit for heralding the start of tissue culture in bamboo goes to Alexander and Rao[27], who reported the aseptic germination of bamboo seeds. Since then, micropropagation through axillary bud proliferation, where no intermediary callus formation occurs, has been largely attempted.

Explant Types

The use of tissue culture as a tool for plant propagation could be particularly relevant for vegetatively propagated crop plants that resist conventional asexual propagation[28] or when mass propagation of single plant is required in short period of time. The different explants, such as, axillary buds, shoot tips, meristem tips, root tips are commonly used. The most common explants used for bamboo micropropagation are young branch node, immature embryos, mature embryos, mesocotyl, leaf sheath, leaf and root of the young seedling.

Explant Disinfection

The main objective behind explants disinfection is to get rid of the bacterial and fungal contamination without hampering the biological activity of the explants. The commonly used sterilants are bleaching, ethanol, sodium hypochlorite (NaOCl), mercuric chloride ($HgCl_2$). The type of sterilant used, its concentration and exposer time depends on the nature of explant and species[29]. The list of various disinfectants used in the tissue culture of bamboo is given in Table 1[27,30-50].

Media and Culture Condition

Types of Media

Media play a vital role in the successful growth and differentiation of excised plant tissues and organs. Murashige and Skoog's (MS)[51] medium was used

Table 1—List of bamboo species with the sterilants, plant growth regulators, organic additives, incubation conditions, acclimatizing material used for its regeneration along with survival rate

Plant species	Sterilant used	PGR	Organic additives	Temp.	Light	Potting mixture	Survival rate	References
Bambusa spp.	-	-	-	76±1ºF	12 h photoperiod	-	-	Alexander and Rao[27]
Dendrocalamus strictus	0.1% $HgCl_2$ for 10 min	BAP, Kn and IAA	Coconut water	25ºC	16 h photoperiod of 1500 lux light intensity	Sterile soil: sand (1:1)	70-80%	Nadgir *et al*[30]
B. glaucescens	20-30% Javex and 5-6% NaOCl	BA and NAA	-	28ºC	14 h photoperiod	Moist sterile soil	-	Banik[31]
Sinocalamus latiflora	70% alcohol for 30 sec and 1% NaOCl solution for 10 min	2,4-D, BA and NAA	-	25±1ºC	16/8 h light/dark cycle with 135 $\mu E/m^2\ s^{-1}$	-	-	Tsay *et al*[32]
Bamboo (54 species)	70% alcohol as spray and 1% NaOCl solution for 10 min	2,4-D, BA and NAA	Coconut water	25ºC	250 µmol $m^{-2}\ s^{-1}$ cool-white fluorescent illumination for 16 h			Prutpongse and Gavinlertvatana[33]
B. vulgaris	1 g dm^{-3} (m/v) Bavistin for 45 min and 0.2% (m/v) $HgCl_2$ solution for 30 min	BA, Kn, 2,4-D and IBA	-	25±2ºC	16 h photoperiod (55 µmol $m^{-2}\ s^{-1}$)	Soil:manure: sand (1:1:1)	90%	Rout and Das[34]
D. asper	4% NaOCl solution for 20 min	BA, NAA and IBA	-	25±1ºC	16 h photoperiod (30 µmol $m^{-2}\ s^{-1}$)	Soil	95%	Arya *et al*[35]
D. giganteus	Bleaching powder for 10 min, Benlate (0.1%) for 1h, 0.3% $HgCl_2$ for 10 min	BA and TDZ	Coconut water	24±2ºC	16h photoperiod	-	-	Ramanayake *et al*[36]
B. edulis	-	BAP, NAA and TDZ	Coconut water	26ºC	16 h photoperiod (54 µmol $m^{-2}\ s^{-1}$)	Peat:vermic-ulite:perlite (1:1:1)	-	Lin *et al*[37]
B. wamin	Wiped with 70% alcohol followed by 0.2% $HgCl_2$ for 5-20 min.	BAP, BA, Kn and IBA	-	25±2ºC	16 h photoperiod (30 µmol $m^{-2}s^{-1}$)	Vermiculite	80-85%	Arshad *et al*[38]
B. vulgaris	0.1% $HgCl_2$ for 20 min	IBA, NAA and BAP	-	25ºC	16 h/8 h photoperiod	-	100%	Ndiaye *et al*[39]
Guadua angustifolia	Extran (0.05% w/v) for 10 min, combination of Agrimycin and Benomyl (@ 2g L^{-1}) for 10 min, NaOCl (1.0 or 1.5% w/v) for 10 min, or with calcium hypochlorite (10% w/v) for 40 min supplemented with a drop of Tween 80 per 100 mL	BAP	-	26ºC	In dark	Soil:sand:rice hulls (1:1:1)	>85%	Jimenez *et al*[40]
B. glaucescens	70% alcohol followed by 5-10 min in 1% w/v Cetrimide and finally in 0.1% $HgCl_2$ for 10 min	BA and Kn	-	25±2ºC	16 h photoperiod (35±10 µmol $m^{-2}\ s^{-1}$)	Soilrite with half strength MS medium (organic free)	80%	Shirin and Rana[41]
B. tulda	0.1% Dettol, 0.2% Bavistin, 70% alcohol followed by NaOCl solution for 10 min	BAP, IAA, Glutamine and Coumarin	-	25±2ºC	16 h illumination (approx 45 µmol $m^{-2}\ s^{-1}$)	Sand:soil: farm yard manure (1:1:1)	100%	Mishra *et al*[42]

Contd.

Table 1—List of bamboo species with the sterilants, plant growth regulators, organic additives, incubation conditions, acclimatizing material used for its regeneration along with survival rate—*Contd.*

Plant species	Sterilant used	PGR	Organic additives	Temp.	Light	Potting mixture	Survival rate	References
D. asper	5% cetavelon for 15 min followed by 0.1% $HgCl_2$ for 7-10 min	BA, NAA and IBA	-	26ºC	16 h photoperiod (3000 µE m^{-2} s^{-1})	Soilrite	95%	Arya *et al*[35]
Arundinaria callosa	0.1% $HgCl_2$ for 10 min	BAP and IBA	-	25±2ºC	-	Soil mixture	60-70%	Devi and Sharma[43]
D. hamiltonii	70% alcohol for 30 sec and 2.5% NaOCl solution for 30 min	2,4-D, BAP, Kn, IBA and NAA	-	25ºC	16 h/8 h photoperiod (30-50 µmol m^{-2} s^{-1})	Potting soil	100%	Zhang *et al*[44]
B. nutans	Tween 20, bavistin (0.1%) and streptomycin sulfate (0.05%) for 20-25 min, 70% ethanol for 1-2 min. Finally (0.04%) $HgCl_2$ with 1-2 drop of liquid detergent for 5-6 min	BAP, NAA and Kn	-	-	14/10h day and night regime (70 ± 5 µmol m^{-2} s^{-1})	Soil and sand (1:1)	90%	Mehta *et al*[45]
B. ventricosa	70% alcohol for 5 min followed by 10% Clorox bleach for 40 min	BAP, Kn, IAA, IBA and NAA	Coconut water	25±2ºC	16-h photoperiod with a light intensity of 47.29 µmol m^{-2} s^{-1}	Pro-mix/ black cinder:potting mixture (1:1)	-	Cheah and Chaille[46]
D. farinosus	70% ethanol for 30 s followed by 0.1% $HgCl_2$ for 30 min	2,4-D, 2,4,5-T, Kn, IAA and IBA	-	25±1ºC	12 h photoperiod (80 µmol m^{-2} s^{-1})	Peat moss, vermiculite and garden soil (2:1:1)	90.1%	Hu *et al*[47]
D. giganteus	20% NaOCl for 20 min	BAP, Kn, IBA, NAA, 2,4-D and GA_3	-	25±2ºC	16-h photoperiod with a light intensity of 90-95 µmol m^{-2} s^{-1}	Sand and soil (1:1)	80-90%	Devi *et al*[48]
D. strictus	1% extran for 10 mins, 0.1% $HgCl_2$ for 5 min, 70% ethanol (1 min)	BAP, Kn, IBA and NAA	-	25ºC	photoperiod of 16 h at 2,000–3,000 lux light intensity of cool white fluorescent tube	Perlite, soil and farm yard manure with a ratio of 1:1:1	70%	Goyal *et al*[49]
D. membranaceous	0.1% Tween 20 for 10 min, bavestin and streptomycin sulphate (0.25% w/v each) for 45 min, 15% NaOCl and 0.1% $HgCl_2$ for 10 min	BAP, Kn and GA_3	-	25±2ºC	16/8 h photoperiod at light intensity of 70 µmol m^{-2} s^{-1} with 75% relative humidity	Sand: soil: farm yard manure (1:1:1)	65%	Brar *et al*[50]

PGR: Plant Growth Regulators; Temp.: Temperature

extensively in the regeneration of bamboos. In cultures of *Dendrocalamus strictus*, Nadgir and his co-workers[30] used Whites basal medium and MS medium for rapid multiplication. They got high rates of multiplication, *i.e.*, approx 10,000 plantlets per seedlings yearly. Tsay and his co-workers[32] used N_6 medium along with MS medium for the regeneration of *Sinocalamus latiflora* and was sucessful in obtaining embryonic calli, while Ndiaye *et al*[39] used three different types of media, *viz.*, MS medium, Gamborg medium and Lloyd and Crown medium, for the rapid proliferation of *Bambusa vulgaris*. Of the three media used, modified MS medium enhanced axillary bud formation and shoot development.

The sugar is supplied in the form of sucrose. Sucrose was added as the source of carbon at a concentration of 3% (w/v) in almost all the experiments. In the culture of *B. glaucescens*[31],

4%sucrose was used for breaking the dormancy of the axillary bud and regeneration, whereas Nadgir *et al*[30] used only 2% sucrose for obtaining multiple shooting. Tsay and his co-workers[32] used different concentration (3, 6, 9 & 12%) of sucrose for their experiment and found higher callus proliferation at sucrose concentration of 9%. Cheah and Chaille[46] used 3% sucrose for *B. ventricosa*. The somatic embryos thus produced developed into a bipolar structure that germinated into complete plantlets with shoot and root systems.

pH of Media

The range of acidity or alkalinity is an important factor that determines the quality of regenerated plantlets from a tissue culture medium. The optimum pH for regeneration varies with the type of explant used. In the cultures of the different bamboo species, pH 5.7-5.8 was generally maintained for their successful regeneration[32,48]. However, Ndiaye *et al*[39] found that pH 5.5-5.6 was suitable for bamboo micropropagation.

Solidification of Media

Generally, all three types of media, *viz*., solid, semisolid and liquid, are used in bamboo tissue culture. However, the medium was solidified with agar 0.8% (w/v) in majority of the cultures[30,32,34,45] with a few exceptions. In case of *B. wamin*[38], 0.75% agar was used; while 0.7% agar agar was used to gell the cultures of *B. glaucescens*[41]. The use of lower concentrations like 0.6% agar was also reported[33]. Gelrite at a concentration of 2 g/L, 2.2 g/L, 3 g/L and 3.5 gL were used as a gelling agent in *B. balcooa*[52], *B. edulis*[37], *B. venticosa*[46] and *B. vulgaris*[39], respectively; while Ogita[53] used 3 g/L gellan gum for *Phyllostachys nigra* and Jimenez and his co-researchers[40] used phytagel (0.2%) for *Guadua anguistifolia* culture.

Plant Growth Regulators

Many researchers preferably term plant hormones as plant growth substances or plant growth regulators. The effect of hormones not only depends on the rate of uptake from the medium, or on the stability in the medium and in the tissue, but also on the sensitivity of the target tissues.

The main plant growth regulators used in tissue culture are auxins, cytokinins, abscisic acid, ethylene etc. A list of the bamboo species and the plant growth regulator used for its regeneration are provided in Table 1. In the regeneration of bamboos, growth regulators like thidiazuron (TDZ), 6-benzylaminopurine (BAP) or benzule adenin (BA), kinetin (Kn), 1-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), 2,4 dichloro-phenoxyacetic acid (2,4-D) etc. are used extensively. Organic additives like coconut water is also employed as supplement in media for the regeneration of bamboos[30,33,37,46,54-56].

Incubation Conditions

Incubation conditions play a great role in plant tissue culture after aseptic inoculation of the explants is done. An optimum temperature is required for obtaining desirable clone. Moreover some tissues may grow in dark, while others prefer light conditions. The amount of light also has substantial effect on tissue regeneration. For instances, Alexander and Rao[27] reported 12 h photoperiodism in *Bambusa* spp., while many workers reported photoperiod as high as 16 h[30,34]. Intensity of light also varies from species to species. We previously reported 2000-3000 lux of cool white florescent light suitable for *D. strictus*[49]. The incubation conditions attempted for bamboos by different workers are also shown in Table 1.

Initiation of Culture

For initiation of callus culture, different parts of the experimental plant like leaf, stem, roots, nodes, etc., containing meristematic cells are used since meristematic cells possess pre-existing growth momentum. In order to obtain embryogenic callus in *B. edulis*, Lin and his co-workers[37] used MS medium supplemented with 9.2 µM Kn, 13.6 µM 2,4-D, 0.1% (v/v) coconut milk in addition to 0.046 µM TDZ. A protocol for callus induction from the shoots of *P. nigra* was developed by Ogita[53]. The cultures produced callus in half strength MS medium supplemented with 3 µM 2,4-D. In *B. vulgaris*, a protocol for producing friable callus was achieved by using *in vitro* sprouted shoots as explants. MS medium supplemented with 2.2 µM BAP, 9.04 µM 2,4-D and 14.76 µM IBA (indole-3-butyric acid) was used for callus initiation[34].

Mehta and her co-workers[45] in their study on *B. nutans* reported the formation of embryogenic calli from *in vitro* sprouted buds in MS medium containing 5 mg/L 2,4-D. Similarly, Cheah and Chaille[46] in their work on *B. ventricosa* also reported the formation of embryogenic calli from *in vitro* developed shoots in MS medium containing 3 mg/L 2,4-D, 2 mg/L Kn.

Hu and his co-researchers[47] employed two different types of explants, *i.e*., mature seeds and young shoots pertaining to induction of callus and plant regeneration of *D. farinosus*. MS medium in combination with 2 mg/L 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), 2 mg/L Kn and 0.4 mg/l IBA gave satisfactory results when mature seeds were used as explants. Callus induction frequency was found to be 95% for mature seeds and 21-29.7% in case of young shoots[49]. In 2012, Devi and coworkers[51] developed a protocol for callus induction and proliferation in edible bamboo *D. giganteus*. MS medium in addition to 3 mg/L 2,4-D and 0.5 mg/Ll Kn was found to be best suited for callusing in *D. giganteus*.

In Vitro Shoot Multiplication

The discovery of growth regulators like auxins, gibberlins, cytokinins and abscicins along with other organic compounds led to new vistas in plant tissue culture. The role of growth regulators and their concentration should, however, be carefully chosen for obtaining desired responses in tissue culture. In *D. strictus* best shoot multiplication and growth was observed in MS medium containing 2 mg/L BAP and 5% coconut milk, where a maximum of 8-10 shoots was observed per flask in liquid culture within 6-7 wk[51].

Ramanayake *et al*[36] observed that axillary buds of *B. vulagris* 'Straita' when cultured in MS medium having 4 mg/L BA resulted in the highest mean shoot number, whereas BA at the conc. of 6 mg/L and TDZ at 0.1 mg/L also produced same number of shoots. Shirin and Rana[41] also reported similar results. In case of *B. balcooa*, BAP at a conc. of 5 mg/L resulted in enormous shoot formation[57].

In Vitro Rhizogenesis

Additional plant growth regulator may or may not be required for *in vitro* rooting in bamboos. Nadgir and his colleagues[30] found that 80% of the shoots in *D. strictus* when treated with IBA prior to culture in MS medium produced roots, while percentage of root production reduced to half in case the plants were not treated with IBA. Embryogenic calli of *S. latifora* was found to produce root when cultured for a long time in MS medium or subcultured in auxin free medium[32]. Similar trends were also observed in case of *B. beecheyana*[58].

Prutpongse and Gavinlertvatana[33] noticed that a concentration of NAA between 2.7 to 5.4 µM was optimal for rooting, depending upon the species of bamboo used. While spontaneous rooting from shoots were reported in *D. asper* with MS medium supplemented with IBA (10 mg/L) and NAA (3 mg/L)[35].

In *B. balcooa*, three different auxins (IBA, NAA & IAA) were used to investigate their effect on rooting. It was found that NAA (6.71 µM) was suitable compared to the other two types of auxins. However, when half strength MS medium was supplemented with different concentrations and combinations of auxins (5.71 µM IAA, 4.9 µM IBA, 5.37 µM NAA), the maximum rooting was evident[52]. The effect of combinations of auxins (0.4 mg/L IBA & 0.25 mg/L IAA) in rooting was also reported in *D. farinosus* by Hu and his co-workers[47]. Recently in 2012, Devi *et al*[48] found that IBA at the concentration of 5 mg/L was optimum for rooting in *D. giganteus*.

Hardening and Acclimatization

After successful *in vitro* regeneration of plantlets in the test tube, major difficulty in transferring the plantlets from laboratory to land remains in the hardening and acclimatization process[59]. This difficulty probably appears due to the drastic difference in environment between test tube and field[60]. Several protocols have been developed by different bamboo culturist to overcome some of these constraints. In *D strictus*, equal amount of soil and sand mixture was proved effective in hardening of plantlets. Banik[31] reported simple moist soil as the best acclimatization medium for *B. glaucescens.* In case of *B. edulis*, Lin *et al*[37] reported peat, vermiculite and perlite at a ratio of 1:1:1 as the most suitable hardening and acclimatization medium, whereas sand, soil and hulls (1:1:1) was preferred mixture of choice for *G. angustifolia*[40] (for details of various hardening mixtures for Bamboos, please refer Table 1).

Conclusion

Bamboo has been the subject of immense curiosity since ages. Traditional breeding in bamboo has been a tough job because of the peculiar flowering patterns and scarce production of seed sets. Thus, *in vitro* regeneration proves to be the best alternative. Though it is impossible to trace out exact mechanism behind *in vitro* regeneration of bamboo, available literature provides a vivid portrait about the different factors governing the *in vitro* regeneration of bamboo. Present review is a humble effort to sum up various ingredients used and techniques employed for successful regeneration of several species of Bamboo.

References

1 Watson L & Dallwitz M J, *The grass genera of the world* (CAB International, Wallingford, Oxon, UK) 1992.

2 Goyal A K, Kar P & Sen A, Advancement of bamboo taxonomy in the era of molecular biology: A review, in *Biology of useful plants and microbes*, edited by A Sen (Narosa Publication House, New Delhi) 2013, 197-208.

3 Ogunjinmi A A, Ijeomah H M & Aiyeloja A A, Socio-economic importance of bamboo (*Bambusa vulgaris*) in Borgu local government area of Niger State, Nigeria, *J Sust Dev Afr*, 10 (2009) 284-289.

4 Goyal A K, Middha S K, Usha T, Chatterjee S, Bothra A K *et al*, Bamboo-infoline: A database for North Bengal bamboo's, *Bioinformation*, 5 (2010)184-185.

5 Goyal A K, Ghosh P K, Dubey A K & Sen A, Inventorying bamboo biodiversity of North Bengal: A case study, *Int J Fund Appl Sci*, 1 (2012) 5-8.

6 Ghosh G K, *Bamboo: The wonderful grass* (APH Publishing, New Delhi, India) 2008, 44.

7 Bansal A K & Zoolagud S S, Bamboo composites: Material of the future, *J Bamboo Rattan*, 1 (2002) 19-130.

8 Satya S, Bal L M, Singhal P & Naik S N, Bamboo shoot processing: Food quality and safety aspect (a review), *Trends Food Sci Technol*, 21 (2010) 181-189.

9 Recht C & Wetterwald M F, Species and cultivars for the garden, in *Bamboos*, edited by D Crampton (B T Batsford, London) 1992, 55-80.

10 Lu B, Wu X, Tie X, Zhang Y & Zhang Y, Toxicology and safety of anti-oxidant of bamboo leaves, Part 1: Acute and subchronic toxicity studies on anti-oxidant of bamboo leaves, *Food Chem Toxicol*, 43 (2005) 783-792.

11 Kweon M H, Hwang H J & Sung H C, Identification and antioxidant activity of novel chlorogenic acid derivatives from bamboo (*Phyllostachys edulis*), *J Agric Food Chem*, 10 (2001) 4646-4655.

12 Zhang Y, Bao B, Lu B, Ren Y, Tie X *et al*, Determination of flavone *C*-glucosides in antioxidant of bamboo leaves (AOB) fortified foods by reversed-phase high-performance liquid chromatography with ultraviolet diode array detection, *J Chromatogr A*, 1065 (2005) 177-185.

13 Park H S, Lim J H, Kim H J, Choi H J & Lee I S, Antioxidant flavone glycosides from the leaves of *Sasa borealis*, *Arch Pharm Res*, 2 (2007) 161-166.

14 Zhang Y, Jiao J, Liu C, Wu X & Zhang Y, Isolation and purification of four flavone *C*-glycosides from antioxidant of bamboo leaves by macroporous resin column chromatography and preparative high-performance liquid chromatography, *Food Chem*, 3 (2008) 1326–1336.

15 Goyal A K, Middha S K & Sen A, *Bambusa vulgaris* Schrad. ex J. C. Wendl. var. *vittata* Riviere & C. Riviere leaves attenuate oxidative stress—An *in vitro* biochemical assay, *Indian J Nat Prod Resour*, 4 (2013) 436-440.

16 Goyal A K, Middha S K & Sen A, Evaluation of the DPPH radical scavenging activity total phenols and antioxidant activities in Indian wild *Bambusa vulgaris* "Vittata" methanolic leaf extract, *J Nat Pharm*, 1 (2010) 40-45.

17 Goyal A K, Middha S K & Sen A, *In vitro* antioxidative profiling of different fractions of *Dendrocalamus strictus* (Roxb.) Nees leaf extracts, *Free Rad Antiox*, 1 (2011) 42-48.

18 Wang J, Yue Y D, Jiang H & Tang F, Rapid screening for flavone *C*-glycosides in the leaves of different species of bamboo and simultaneous quantitation of four marker compounds by HPLC-UV/DAD, *Int J Anal Chem*, 2012 (2012) 205101.

19 Janzen D H, Why bamboos wait so long to flower, *Annu Rev Ecol Evol Syst*, 7 (1976) 347-391.

20 Weathers P J, Towler M J & Xu J, Bench to batch: Advances in plant cell culture for producing useful products, *Appl Microbiol Biotechnol*, 85 (2010) 1339-1351.

21 Banik R L, *A manual for vegetative propagation of bamboos* (International Network for Bamboo and Rattan, Beijing, China) 1995.

22 Bisht P, Pant M & Kant A, *In vitro* propagation of *Gigantochloa atroviolaceae* Widjaja through nodal explants, *J Am Sci*, 6 (2010) 1019-1025.

23 Murashige T, Plant propagation through tissue cultures, *Annu Rev Plant Physiol*, 25 (1974) 135-166.

24 Evans D A, Sharp W R & Flinck C E, Growth and behaviour of cell cultures: Embryogensis and organogenesis, in *Plant tissue culture: Methods and applications in agriculture*, edited by T A Thrope (Academic Press, New York) 1981, 45-114.

25 Rahman M M, Amin M N, Ahamed T, Ali M R & Habib A, Efficient plant regeneration through somatic embryogenesis from leaf base derived callus of *Kaempferia galanga* L., *Asian J Plant Sci*, 3 (2004) 675-678.

26 Hartmann H F, Kester D E, Dauies F D Jr & Geneve R L, *Plant propagation—Principles and practices*, 6th edn (Prentice Hall of India Private Ltd, New Delhi, India) 1997, 549-611.

27 Alexander M P & Rao T C, *In vitro* culture of bamboo embryos, *Curr Sci*, 37 (1968) 415.

28 Hackett W P, Application of tissue culture to plant propagation, in *16th Proc Int Plant Propagator's Society* (Carlisle, USA) 1966, 88-92.

29 Razdan M K, *Introduction to plant tissue culture* (Science Publishers Inc., New Hampshire, USA) 2003, p 330.

30 Nadgir A L, Phadke C H, Gupta P K, Parsharami V A, Nair S *et al*, Rapid multiplication of bamboo by tissue culture, *Silvae Genet*, 33 (1984) 219-233.

31 Banik R L, Techniques of bamboo propagation with special reference to pre-rooted and pre-rhizomed branch cuttings and tissue culture, in *Proc Int Bamboo Workshop on Recent Research on Bamboos* (IDRC, Hanngzhou, China) 1985, 160-169.

32 Tsay H S, Yeh C C & Hsu J Y, Embryogenesis and plant regeneration from anther culture of bamboo (*Sinocalamus latiflora* (Munro) McClure), *Plant Cell Rep*, 9 (1990) 349-351.

33 Prutpongse P & Gavinlertvatana P, *In vitro* micropropagation of 54 species from 15 genera of bamboo, *HortScience*, 27 (1992) 453-454

34 Rout G R & Das P, *In vitro* plant regeneration *via* callogenesis and organogenesis in *Bambusa vulgaris*, *Biol Plant*, 39 (1997) 515-522.

35 Arya S, Satsangi R & Arya I D, Direct regeneration of shoots from immature inflorescences in *Dendrocalamus asper* (edible bamboo) leading to mass propagation, *Bamboo Sci Cult*, 21 (2008) 14-20.

36 Ramanayake S M S D, Meemaduma V N & Weerawardene T E, *In vitro* shoot proliferation and enhancement of rooting for the large-scale propagation of yellow bamboo (*Bambusa vulgaris* 'Striata'), *Sci Hortic*, 110 (2006) 109-113.

37 Lin C-S, Lin C-C & Chang W-C, Effect of thidiazuron on vegetative tissue-derived somatic embryogenesis and flowering of bamboo *Bambusa edulis*, *Plant Cell Tissue Organ Cult*, 76 (2004) 75-82.

38 Arshad S M, Kumar A & Bhatnagar S K, Micropropagation of *Bambusa wamin* through proliferation of mature nodal explants, *J Biol Res*, 3 (2005) 59-66.

39 Ndiaye A, Diallo M S, Niang D & Gassama-Dia Y K, *In vitro* regeneration of adult trees of *Bambusa vulgaris*, *Afr J Biotechnol*, 5 (2006) 1245-1248

40 Jiménez V M, Castillo J, Tavares E, Guevara E & Montiel M, *In vitro* propagation of the neotropical giant bamboo *Guadua angustifolia* Kunth, through axillary shoot proliferation, *Plant Cell Tissue Organ Cult*, 86 (2006) 389-395.

41 Shirin F & Rana P K, *In vitro* plantlet regeneration from nodal explants of field-grown culms in *Bambusa glaucescens* Willd., *Plant Biotechnol Rep*, 1 (2007) 141-147.

42 Mishra Y, Patel P K, Yadav S, Shirin F & Ansari S A, A micropropagation system for cloning of *Bambusa tulda* Roxb., *Sci Hortic*, 115 (2008) 315-318.

43 Devi W S & Sharma G J, *In vitro* propagation of *Arundinaria callosa* Munro—An edible bamboo from nodal explants of mature plants, *Open Plant Sci J*, 3 (2009) 35-39.

44 Zhang N, Fang W, Shi Y, Liu Q, Yang H *et al*, Somatic embryogenesis and organogenesis in *Dendrocalamus hamiltonii*, *Plant Cell Tissue Organ Cult*, 103 (2010) 325-332.

45 Mehta R, Sharma V, Sood A, Sharma M & Sharma R K, Induction of somatic embryogenesis and analysis of genetic fidelity of *in vitro*-derived plantlets of *Bambusa nutans* Wall using AFLP markers, *Eur J Forest Res*, 130 (2011) 729-736.

46 Cheah K T & Chaille L C, Somatic embryogenesis from mature *Bambusa ventricosa* (College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu, USA) 2011, Biotechnology, Bio-11.

47 Hu S L, Zhou J Y, Cao Y, Lu X Q, Duan N *et al*, *In vitro* callus induction and plant regeneration from mature seed embryo and young shoots in a giant sympodial bamboo *Dendrocalamus farinosus* (Keng et Keng f.) Chia et H. L. Fung, *Afr J Biotechnol*, 10 (2011) 3210-3215.

48 Devi W S, Bengyella L & Sharma G J, *In vitro* seed germination and micropropagation of edible bamboo *Dendrocalamus giganteus* Munro using seeds, *Biotechnology*, 11 (2012) 74-80.

49 Goyal A K, Pradhan S, Basistha B C & Sen A, Micropropagation and assessment of genetic fidelity of *Dendrocalamus strictus* (Roxb.) Nees using RAPD and ISSR markers, *3Biotech*, 5 (2015) 473-482.

50 Brar J, Anand M & Sood A, *In vitro* seed germination of economically important edible bamboo *Dendrocalamus membranaceous* Munro, *Indian J Exp Biol*, 51 (2013) 88-96.

51 Murashige T & Skoog F, A revised medium for rapid growth and bioassay with tobacco tissue cultures, *Plant Physiol*, 15 (1962) 473-497.

52 Negi D & Saxena S, Micropropagation of *Bambusa balcooa* Roxb. through axillary shoot proliferation, *In Vitro Cell Dev Biol-Pantl*, 47 (2011) 604-610.

53 Ogita S, Callus and cell suspension culture of bamboo plant *Phyllostachys nigra*, *Plant Biotechnol*, 22 (2005) 119-125.

54 Nadgauda R S, Parasharami V A & Mascarenhas A F, Precocious flowering and seeding behaviour in tissue-cultured bamboos, *Nature* (*Lond*), 344 (1990) 335-336.

55 Ramanayake S M S D, Wanniarachchi W A V R & Tennakoon T M A, Axillary shoot proliferation and *in vitro* flowering in an adult giant bamboo *Dendrocalamus giganteus* Wall. ex Munro, *In Vitro Cell Dev Biol-Plant*, 37 (2001) 667-671.

56 Nadgauda R S, John C K, Joshi M S, Parasharami V A & Mascarenhas A F, A comparison of *in vitro* with *in vivo* flowering in bamboo: *Bambusa arundinacea*, *Plant Cell Tissue Organ Cult*, 48 (1997) 181-188.

57 Mudoi K D & Borthakur M, *In vitro* micropropagation of *Bambusa balcooa* Roxb. through nodal explants from field-grown culms and scope for upscaling, *Curr Sci*, 96 (2009) 962-966.

58 Chang W-C & Lan T-H, Somatic embryogenesis and plant regeneration from roots of bamboo (*Bambusa beecheyana* Munro var. beecheyana), *J Plant Physiol*, 145 (1995) 535-538.

59 Wardle K, Dobbs E B & Short K C, *In vitro* acclimatization of asceptically cultured plantlets to humidity, *J Am Soc Hortic Sci*, 108 (1983) 386-389.

60 Desjardins Y, Gosselin A & Yelle S, Acclimatization of *ex vitro* strawberry plantlets in CO_2-enriched environments and supplementary lighting, *J Am Soc Hortic Sci*, 112 (1987) 846-852.