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Factors Influencing Micropropagation Of Bamboo Species Using Nodal Explants: A Review.

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ABSTRACT

Micro propagation has played a very significant role in rapid multiplication of different species of bamboo to meet the global need of disease free saplings. Commendable researches have been carried out on the micro propagation using apical buds or nodal segments in last few decades. Individual research focused to understand the specific requirement at different stages of micro propagation technique. Protocol standardization to maximize rate of shoot multiplication through cost effective micro propagation gained much importance. A huge data were generated and numbers of papers have been published on several factors influencing *in vitro* organogenesis of bamboo. The present review was aimed to give a comprehensive account on factors influencing micro propagation of bamboo based on the findings of previous literatures. It was evident that nodal segment is the best explant which further depends on its position, collection time and species. BA is the widely used shooting hormone and the use of combination of auxins as rooting hormone, is advantageous.

Keywords: bamboo, nodal explants, *in vitro* propagation

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INTRODUCTION

Perennial grasses with large woody stem or culm belonging to subfamily Bambusoideae under family Poaceae is taxonomically known as bamboo (Chaowana 2013). This plant is considered as the most stable (Shukla et al. 2012), versatile and fastest growing (Roy et al. 2014) woody plants around the globe.

Its multipurpose usability from “cradle to coffin” made bamboo “green gold” (Bhattacharyya et al. 2010). In India, the projected export potential is amounting to Rs.30, 000 crores within 2025 (Mehra and Mehra 2007). National Institution for Transforming India (NITI) Ayog (erstwhile Planning commission), Government of India, estimated that domestic bamboo available in India is sufficient to fulfil only 50% demands of the whole country (Times of India, dated 8 April 2016). It is pertinent to explore the suitable method for rapid and large scale propagation method to meet the gap between demand and supply.

Bamboo is traditionally propagated by vegetative method (Ahlawat et al. 2002) as seed based propagation is very limited due to its long flowering cycle, low seed viability, seed sterility etc (Singh et al. 2013). But large plantation through vegetative method like offset cutting, rhizome cutting etc but very difficult due to bulkiness of cutting materials (Sharma and Sarma 2013), seasonal dependency and low rooting capacity (Bakshi et al. 2015). Micropropagation is considered as the most reliable source to season independent supply of large amount of true to the type plants within reasonably short period of time (Aitken-Christie et al. 1995) and would help in multiplication as well as in conserving wild germ-plasm (Mudoj et al. 2013).

Micro-propagation in Bamboo:

Several types of explants i.e; seed, inflorescences, stem-node sections, meristem domes or leaves etc (Prutpongse and Gavinlertvatana 1992) are till now reported for bamboo micro propagation. Among them the nodal explants are considered as the most efficient explants for its active meristem and capacity to produce axillary buds which converts into plantlets (Goyal et al. 2015). The response of node again depends on the collection of time (season) from field, its physiological status (Ramanayake and Yakandawala 1997) and its position in mother plant (Chowdhury et al. 2004). Genotype and physiological stage of node to be used varies species to species. In bamboo, both juvenile and mature plants can be considered as explants (Malini and Ananda kumar 2013). Though both direct and indirect methods, for organogenesis and somatic embryogenesis, are reported for in vitro propagation of bamboo but most of research work till now reported on direct organogenesis using nodal explants/auxillary bud since its reduce the chance of somaclonal variation (Singh et al. 2013). In the present review, a consolidated account is dealt on different factors affecting direct organogenesis using auxiliary bud or nodal segment in bamboo, based on huge data generated during last few decades.

Factors related to direct organogenesis using Auxiliary bud/node:

Explant: For higher regeneration, proper selection of suitable explants that depends on several factors like plant type, objective of study and type of culture (George et al. 2008), is very critical to induce the bud breaking in nodal explants. Larger explants (25 mm) are more suitable than smaller one (5-7 mm) to initiate the culture within short time because of its high endogenous hormonal effect (Anand et al. 2013) for *Bambusa bambos*.

Collection time of explants: The success in micropropagation is the absolute interaction of species and seasons. It depends on variation in external contamination or in the internal concentration of plant hormones (George et al. 2008). Collection of nodal explants during pre-monsoon season promotes maximum response in *Dendrocalamus asper* (Singh et al. 2012b). Rainy season was found better in *Bambusa tulda* (Mishra et al. 2008), *Gigantochloa atroviolaceae* (Bisht et al. 2010), *D. strictus* (Chaturvedi et al. 1993) which contradicts the findings of Mehta et al. (2011) for *B. nutans*. In spite of high bud breaking, high rate of contamination also found in rainy season reported by several authors (Nadha et al. 2013, Mehta et al. (2011) and Mishra et al. 2008).

Position of nodal explants: Very few works till are executed to draw a valid conclusion regarding nodal position in stock plant. Though Saxena and Bhojwani (1993) reported that node from mid of lateral branches found effective for *D. longispathus* but Chowdhury et al. (2004) reported that node collected from 1st and 2nd position from base of secondary branches had the best regeneration capacity during in vitro propagation of *D. strictus*. Explants collected from middle node of culm found effective for in vitro propagation of *B. vulgaris* (Hirimburegama and Gamage 1995). Devi and Sharma (2009) reported that nodal explants of the top position had low bud-break compared to the basal and mid-culm nodes of the secondary branches in *Arundinaria callosa* Munro. So, nodal segment from the top of branches are least suitable for bamboo micropropagation.

Surface sterilization: Before culture initiation, the explants are surface sterilized by using specific concentration of a surface sterilant for a certain period of time to get the decontaminated plants. Among the surface sterilants, Mercuric Chloride ($HgCl_2$) was found effective over others surface sterilants (Sodium hypochloride, Potassium Hypochloride, Hydrogen Peroxide etc.) reported by several authors for bamboo micropropagation though duration may vary species to species. $HgCl_2$ (0.1%) was found superior over Sodium Hypochloride in *B. tulda* (Mishra et al. 2008); Calcium Hypochloride in *D. strictus* (Pandey and Singh 2012) and Sodium hypochloride and Hydrogen peroxide in *B. ventricosa* was reported by Wei et al. (2015).

Though high concentration (0.2%) of mercuric chloride increased the chance of aseptic culture in *D. strictus* (Kapruwan et al. 2014), but negative impact of this chemical found on bud breaking percentage of plant reported in *B. wamin* (Arshad et al. 2005). On the contrary, Wei et al. (2015) was reported that lower duration of 0.1% $HgCl_2$ increases high survival rate in *B. ventricosa*. Increase of duration of 0.1% $HgCl_2$ is able to control both of fungal and bacterial contamination in *D. strictus* (Goyal et al. 2015). Though Bavistin was reported as effective fungicide in *G. atroviolaceae* (Bisht et al. 2010) but Benomyl (systematic fungicide) is also reported as effective surface sterilant for several bamboo species (Chowdhury et al. 2004; Ramanayake et al. 1995). This entire chemical fruitfully eradicates the surface contaminations but has limitation in controlling the endophytic contamination. Now a day, the antibiotics are also being used during surface sterilization. Compromisation among concentration and duration of the surface sterilant used for micro-propagation with bud breaking percentage is required by the researchers.

Medium and solidifying agent: First successful bamboo tissue culture was reported by Alexander and Rao (1968) in *D. strictus* using Huang and Murashige medium (Huang and Murashige 1983; Sharma and Sarma 2011). For getting maximum auxillary bud breaking, MS (Murashige and Skoog 1962) medium has been proved to be more responsive for both direct as well as indirect organogenesis in bamboo as evident from the study of Singh et al. (2012b) in *D. asper*; Ndiaye et al. (2006) in *B. vulgaris*; Goyal et al. (2015) in *D. strictus*; Saxena and Bhojwani (1993) in *D. longispathus*, Kurz; Saxena (1990) in *B. tulda* in comparison to other available media such as SH (Schenk and Hildebrandt 1972), B5 (Gamborg et al. 1968) and NN (Nitsch and Nitsch 1969), WP Medium (Lloyd and Crown 1980). Though most of the experiments were carried out in semi-solid/solid MS but liquid media also reported by several authors (Table 1). Liquid medium was found more suitable for high bud breaking in *B. Wamin* (Arshad et al. 2005); shoot proliferation in *B.*

oldhamii (Lin et al. 2007) than solid medium. Besides, found high bud breaking in liquid MS than semi soild MS for; Patel et al. (2015) for *B. Balcooa* Roxb; Kavitha and Kiran (2014) in *D. brandisii* Kurz. It may be due to lack of agar in media increase the uptake of nutrients (Bag et al. 2000). Though the multiplication rate of bamboo improved under liquid media but major limitation of its use is the hyperhydration of shoots (Negi and Saxena 2011).

In general 0.8% agar is widely used for bamboo micro-propagation. Agar is most popular as solidifying agent in plant tissue culture due to its stability unique gelling property and not influences the plant metabolism (Ozel et al. 2008). Beside agar, geltrite (0.2-0.35%) and phytogel (0.2%) is also reported as solidifying agent for bamboo tissue culture by several authors (Table 2). The geltrite, another solidifying

agent had several advantages over agar like its clarity enabling faster detection of contamination at wide range of P^H (Bhojwani and Razdan 1996).

Plant Morphogenesis and Plant Growth Regulator: Phytohormones are the chemicals, which at very low concentration are capable to modify the growth and morphogenesis of plant (George et al. 2008). Synthetic compounds acting like plant growth hormones are termed as plant growth regulators (Davies 1995; Gasper et al. 1996). Cytokinin and auxin are most widely used respectively for caulo- and rhizo- genesis during plant tissue culture.

Shoot induction and proliferation/ multiplication: In bamboo tissue culture, 6-Benzylaminopurine (6-BAP) or 6-BA (Benzyl Adenine) is widely used for shoot proliferation which might be due to its cost effectiveness and autoclavable nature (Goyal et al. 2015). In terms of bud breaking and shoot proliferation, BAP/BA was found more effective than other cytokinins for several bamboo species including *B. nutans* (Sharma and Sarma 2014), *B. tulda* (Sharma and Sarma 2013), *D. asper* (Nadha et al. 2013), *D. strictus* (Nadgir et al. 1984; Goyal et al. 2015), *Gigantochloa atroviolaceae* (Bisht et al. 2010) etc. Though use of Kinetin (Kin) is still limited but synergistic effect of both of BAP/BA and Kin was found best for shoot induction and multiplication breaking in several bamboo species including *D. giganteus* Munro (Ramanayake and Yakandawala 1997); *Bambusa arundinacea* Retz. Willd (Kalaiarasi et al. 2014), *B. nutans* (Negi and Saxena 2011), *B. tulda* (Das and Pal 2005a) etc.

Recently, Thidiazuron (TDZ), another cytokinin is gaining importance and TDZ based micropropagation was reported in *B. edulis* (Lin and Chang 1998), *B. oldhamii* Munr (Lin et al. 2007), *D. strictus* (Singh et al. 2001). Comparison among the cytokinins, TDZ was found superior over Kin and BAP in *D. hamiltonii* (Singh et al. 2012a). In vitro propagation of *B. Oldhamii* by Lin et al. (2007) concluded TDZ was the stable and effective cytokinin for proliferation in long-term subcultures. The synergistic effect of TDZ with BAP was studied by Kapruwan et al. (2014) for *D. strictus* and found better bud breaking and shoots proliferation. But TDZ was found ineffective at high concentration (1mg/l and more) for *B. tulda* (Das and Pal 2005b). In addition these, cytokinin TDZ also reported for bamboo multiplication but its use is very limited. The use of TDZ for multiplication are reported in *B. edulis* (Lin and Chang 1998), *D.hamiltonii* (Singh et al. 2012a); *D. strictus* (Kapruwan et al. 2014). Since further use of this phytohormone promote pseudoshoots and that is why MS liquid medium with BAP was found to be suitable for further shoot multiplication and maintenance of bamboo culture (Kavitha and Kiran 2014).

Addition of auxins to multiplication media is reported by Devi and Sharma (2009) for *Arundinaria callosa* Munro. They found IBA was superior over NAA for shoot multiplication. Besides, Gibberellic acid (GA) was also found effective and enhance multiple shoot production in *B. vulgaris* (Hirimburegama and Gamage1995). Limited report is available on the use of auxin for shooting along with cytokinins. NAA coupled with BAP were reported in *B. balcooa* and *B. bambos* (Rathore et al. 2009). Details about the phytohormones with effective concentration for direct organogenesis on different bamboo species using the nodal explants are given in Table 3.

Rooting: Cluster of three or more shoots is most suitable for transferring shoots into rooting medium (Singh et al. 2012b; Nadha et al. 2013). Both Full strength and half strength MS coupled with rooting hormone are reported for rooting (Table 1). Though Kavitha and Kiran (2014) was reported that liquid $\frac{1}{2}$ MS is more suitable for inducing rooting in *D. Brandisii* Kurz than solid $\frac{1}{2}$ MS but Goyal et al. (2015) reported full MS more effective than $\frac{1}{2}$ MS for rooting in *D. strictus*.

Generally three auxins (IAA, NAA and IBA) are widely used for inducing roots in vitro. It is reported that autoclavable IBA is more stable than IAA in liquid medium under growth chamber (Nissen and Sutter 1990) with exception in few species. The limitation of IBA is its low rooting percentage in case of matured explants (Paranjthyet al. 1990). Several authors observed half MS supplemented with NAA (1 - 3 mg/l) is better than that of IBA (Islam and Rahman 2005; Sharma and Sarma 2013; Kavitha and Kiran 2014; Mudoj et al. 2014) in several bamboo species. Though contradictory reports are available regarding this point. These three auxins were found effective for rooting in *D. asper* (Kumar and Banerjee

2014) at low concentration (1mg/l) and IBA was the best for root induction. IBA found superior over NAA; reported in *B. arundinacea* (Venkatachalam et al. 2015); *D. giganteus* (Yasodha et al. 2010). Though only 20 % rooting was achieved using IBA, while IAA, NAA became ineffective for *D. strictus*, (Pandey and Singh 2012). Singh et al. (2012b) observed synergistic effect of both IBA with NAA in place of their single use in medium is better for rooting in *D. asper*. Moidui and Borthakur (2009) reported combination of NAA (3 mg/l) and BAP (1 mg/l) were found most effective in rooting of *B. balcooa* which was in conformity with Goyal et al. (2015) for *D. strictus*. Report regarding IAA alone as rooting hormone for bamboo tissue culture is very limited. Kapruwan et al. (2014) reported IAA for rooting of *D. strictus*. Effect of growth regulators on rooting also vary species to species and also depend upon age of explants (Ramanayake et al. 2008). Beside various auxins, cucurmin also reported for rooting in *D. giganteus* (Ramanayake and Yakandawala 1997), *D. longispathus* KURZ (Saxena and Bhojwani 1993), *B. tulda* and *Melocanna baccifera* (Waikhom and Louis 2014). Details about effective rooting hormones are given in Table 3.

Multiplication: In most cases during bamboo tissue culture, propagules having more than 2-3 shoots are transferred to liquid medium for multiplication after 3-4 weeks of culture initiation. Sub-culturing of shoots is usually done at 3 to 4 weeks interval to prevent the browning the plant and increase the culture (Mudoj and Borthakur 2009; Bisht et al. 2010). But continuous culture on liquid medium leads to verification of plant and multiplication rate reduced. This problem may overcome by adding phytoigel in media to get vitrification free culture (Bag et al. 2000).

Multiplication of bamboo depends on several factors i.e; type of medium, propagule size, its orientation in vessel and number of shoots per propagule. Liquid MS was found more effective than semi solid MS for multiplication as reported in *D. asper* by Banerjee et al. (2011), *D. brandisii* by Kavitha and Kiran (2014). It may be due to high uptake of minerals and hormones by plant enhanced multiplication rate under liquid media (Arshad et al. 2005). Contradictory report was done by Nadha et al. (2013) for *D. asper*. Though kin found less effective but synergistic effect of both cytokinins with addition to adenine sulphate promotes best results in multiplication of bamboo (see plant growth regulator and the Table 1.)

The propagules having more than 2-3 shoots are found more effective in several bamboo species in *D. strictus* (Pandey and Singh 2012); *B. tulda* (Sharma and Sarma 2013); cluster of four shoots are suitable in *D. hamiltonii* (Godbole et al. 2002).

Liquid MS with BAP 3 mg/l is most suitable for bamboo multiplication. More than 3 mg/l BAP declined the multiplication of *B. arundinacea* Retz. Willd (Kalaiarasi et al. 2014). Mudoj and Borkhadur (2009) were found increase of BAP concentration may increase the multiplication but leaves of plants became shorter and more condense. This may be due to over dose of hormone which is toxic for the plant leading to smaller and abnormal leaves. Though shoot multiplication at more than 3 mg/l BAP reported in *Thamnocalamus falconeri*, Hook.f. ex Munro (Bakshi et al. 2015).

Hardening: Lab to land transfer of in vitro propagated plant is another big challenge of micro-propagation. Tissue culture plants are grown under heterotrophic nutrition, unable to survive in the environmental changes due to lack of appropriate hardening and adaptation (Patel et al. 2015). Plants having leaves with less cuticular wax, improper stomatal mechanism, coupled with low photosynthetic activity make itself vulnerable (Singh et al. 2013). Direct transfer of in vitro propagated plantlets to external environment increases mortality percentage of plants because of their inability to survive against biotic and abiotic stresses (Deb and Imchen 2010). Direct sunlight is harmful for plantlets; it leads to wilting of plants (Bhajwani and Razdan 1996). So, an efficient and effective hardening protocol for in vitro propagated plantlets is needed for acclimatization in vivo.

In general, the healthy rooted plantlets are removed from the rooting medium and transferred to pot containing growth supporting materials like soil, sand, soilrite, perlite, vermiculite, compost, farm yard manure etc either alone or in various ratios.

In general, the healthy rooted plantlets are removed from the rooting medium and transferred to pot containing growth supporting materials like soil, sand, soilrite, perlite, vermiculite, compost, farm yard manure etc either alone or in various ratios. Out of several substrate used, soil: sand: farmyard manure (1:1:1) are widely used. This treatment is reported in almost all bamboo species namely, *B. bambos* (Anand et al. 2013), *D. asper* (Arya et al. 2002); *B. balcooa* (Gantait et al. 2016), *G. atroviolaceae* (Bisht et al. 2010), *D. strictus* (Kapruwan et al. 2014). The same substrate with modified ratio was reported by several workers; soil: sand: cow dung in a ratio of (1:1:2) reported by Mudoi et al. (2014), Sharma and Sarma (2014) in *B. nutans*, soil mixture of peat, perlite, and vermiculite (1:1:1) in *B. oldhamii* (Lin et al. 2007), soil: sand: composted coir pith (1:1:1) in *B. nutans* (Yasodha et al. 2008); perlite, soil and farm yard manure (1:1:1) in *D. strictus* (Goyal et al. 2015); 3:1 ratio of coco peat and vermicompost (3:1) in *B. balcooa* (Patel et al. 2015) have also been reported. During first phase of hardening, the plantlets are transferred to pot mixer and irrigated with water (Islam and Rahaman 2005; Sharma and Sarma 2014) or liquid 1/2 MS (Arya et al. 2002; Singh et al. 2012a) or Knops solutions (Pandey and Singh 2012). During this phase, first 20 days (primary hardening) high humidity is required to plant so that they are protected from shock. To keep high humidity, the plants are covered with plastic during that phase. Thereafter the humidity is gradually reduced after one month. After that, plants are transferred to polybags and transferred to green house for further growth (Secondary hardening) and maintained until field transfer. More than 90 % survival rate of several bamboo species are reported in *D. asper* (Arya et al. 1999; Banerjee et al. 2011; Singh et al. 2012b); *D. hamiltonii* (Singh et al. 2012a), *B. tulda* (Mishra et al. 2011); *B. balcooa* (Mudoi and Borkhadur 2009; Patel et al. 2015), *B. nutans* (Negi and Saxena 2011).

Problems in bamboo micro propagation: In vitro contamination of bacteria (Abdulminam et al. 2009) and fungus (Pal and Das 2005a) is causing undesirable effect on the growth of plants. Endophytic contamination that occurred more when explant is collected from mature mother stock is very challenging to control unlike epiphytic contamination (Gielis and Oprins 2002). To reduce endo phytic contamination, efficient antibiotics with proper dose and duration depending on species, is suggested to use as surface sterilants discussed earlier (see surface sterilization part). Phenolic oxidation due to rupturing of cell during excision of cells leads to browning of explants is considered as common phenomenon in tissue culture of woody species (Negi and Saxena 2011). This problem may be reduced by several ways including addition of polyphenol adsorbents like activated charcoal or ascorbic acid (Arditti and Ernst 1993), PVP (Laine and David 1994;) or silver nitrate (Sanyal et al. 2005) to the culture medium. Among them addition of antioxidant to medium was found more effective than others to get rid of the browning problem during micro propagation of Bamboo (Mudoi et al. 2013).

CONCLUSION

Large scale production of quality planting materials through micro propagation is the need of the hour to compensate the natural bamboo stands destroying everyday by human activities. For increasing the regeneration capacity, one must consider several factors discussed in the present review. Bamboo nodal explants, being the best explants, showed differential responses to collection time and its position and species of mother plant. It was evident that most of the research work till now restricted to BA/BAP for shoot induction. Several studies indicated that the use of most promising cytokinin TDZ may be expedited in bamboo. In vitro rooting in bamboo is species specific and combinations of rooting hormone are preferred. The hardening procedure is restricted to use of sand while other substrates may be taken into consideration to increase survivality. The future research must address those issues and will be able to serve better way to generate huge no of elite planting materials within short period of time.

Table 1: Types of media for shoot and root induction in bamboo.

Stage	Semisolid MS	Half strength MS	Liquid MS
Culture initiation	<i>D. giganteus</i> (Ramanayake and Yakandawala 1997); several bamboo species of genus <i>Bambusa</i> and <i>Thyrsostachys</i> (Islam and	<i>Thamnocalamus</i> <i>spathiflorus</i> (Trin.) (Bag et al. 2000); <i>D. hamiltonii</i> Nees	<i>Bambusa tulda</i> Roxb (Saxena 1990); <i>Arundinaria callosa</i> (Devi and Sharma 2009),

	Rahman 2005); <i>B. vulgaris</i> , <i>D. giganteus</i> and <i>D. strictus</i> (Rout and Das 1994); <i>B. oldhamii</i> Munro (Lin et al. 2007).	(Godbole et al. 2002).	<i>Phyllostachys meyeri</i> McClure (Ogita et al. 2008), <i>G. atroviolaceae</i> Widjajan (Bisht et al. 2010), <i>B. oldhamii</i> Munro (Thiruvengadam et al. 2011).
Rooting	Half strength MS	Full strength MS	
	<i>B. wamin</i> (Arshad et al. 2005), <i>B. arundinacea</i> (Kalaiarasi et al. 2014), <i>B. nutans</i> (Negi and Saxena 2011), <i>B. balcooa</i> (Negi and Saxena 2011), <i>D. strictus</i> Nees (Chowdhury et al. 2004), <i>D. hamiltonii</i> (Sood et al. 2002).	<i>B. balcooa</i> (Mudoi and Borkhder 2009), <i>Dendrocalamus strictus</i> Nees (Ravikumar et al.1998; Pandey and Singh 2012); <i>D. hamiltonii</i> (Agnihotri and Nandi 2009).	

Table 2: Solidifying agent for bamboo media (Phytogel and Gelrite)

Phytogel as solidifying agent	Gelrite as solidifying agent
<i>B. bambos</i> (Anand et al. 2013), <i>D. hamiltonii</i> (Agnihotri and Nandi 2009), <i>T. spathiflorus</i> (Bag et al. 2000).	<i>B. balcooa</i> (Negi and Saxena 2011) ; <i>B. edulis</i> (Lin and Chang 1998); <i>B. tulda</i> (Sharma and Sarma 2013); <i>B. ventricosa</i> (Cheah and Chaille 2011); <i>B. vulgaris</i> (Ndiye et al. 2006)

Table 3: Bamboo tissue culture using the nodal explants

Bamboo Species	Bud breaking	Shoot Multiplication	Rooting	Reported by
<i>Arundinaria callosa</i> Munro	MS (L)+ 13.3 µM (BAP)	Liquid MS (L) + 13.3 µM BAP +1.0 µM IBA	1/2 MS (L) + 25 µM IBA + 0.05 µM BAP	Devi and Sharma (2009).
<i>Bambusa arundinacea</i> Retz. Willd	MS + 3.0 mg/l (BAP) + 0.5 mg/l (KIN)	MS + 3.0 mg/l (BAP) + 0.5 mg/l KIN	½ MS+ 2.0 mg/l (IBA) + 0.5 mg/l (KIN)	Kalaiarasi et al. (2014)
<i>B. bambos</i>	MS + 4.4 µM (BAP)	MS+ 4.4 µM (BAP) + 1.16 µM (Kin)	MS+ 9.80 µM IBA	Anand et al. (2013)
<i>B. balcooa</i> Roxb	MS+ 25 mg/l citrate + 50 mg/l ascorbate + 3.5 mg/l (BAP)	MS+ 3 mg/l (BAP) + 0.5 mg/l (NAA)	MS + 4 mg/l NAA	Patel et al. (2015)
<i>B. balcooa</i> Roxb.	MS+ 0.1 mg/l (TDZ)+ 2g/l Gelrite	MS + 0.1 mg/l (TDZ)	MS + 0.01 mg/l (TDZ)+ 0.5 mg/l (2,4-D).	Lin and Chang (1998)
<i>B. balcooa</i> Roxb.	MS + 1 mg/l (BAP)	MS+1 mg/l (BAP)	MS+1 mg/l BAP + 3mg/l NAA	Mudoi et al. (2009)
<i>B. balcooa</i>	MS+ 4 mg/l (BAP)	MS (L)+ 4 mg/l (BAP)	MS (L)+ 1mg/l (IBA)	Gantait et al. (2016)
<i>B. balcooa</i>	Liquid MS + 1 mg/l (BAP)	MS+ 1.0-5.0 mg/l (BAP)	½ MS+3 mg/l (NAA)/5 mg/l (IBA)	Islam and Rahman (2005)
<i>B. balcooa</i>	MS (L) + 11.25 µM (BAP) + 4.5 µM (kin)	MS (L) + 1 µM (IBA)	½ MS (L) + 1 µM (IBA)	Das and Pal (2005a)
<i>B. balcooa</i>	MS+ 4.4 µM (BAP) + 2.32 µM (Kn)+0.2% w/v gelrite	MS (L) + 6.6 µM (BAP)+ 2.32 µM (Kn)+ 2.5% (v/v) coconut water+100mg /l myoinositol	1/2 MS + 5.71 µM (IAA)+ 4.9 µM (IBA) + 5.37 µM (NAA)	Negi and Saxena (2011)
<i>B. glaucescens</i> Willd	MS + 5 µM BA	Liquid MS+ 5 µM BA + 15 µM Kn	MS+ 25 µM IBA	Shirin and Rana (2007)
<i>B. nutans</i> Wall ex. Munro	MS+ 1.0 mg/l(BAP)	MS + 0.5 mg/l (BAP) + 0.1 mg/l (NAA).	MS+2.0 mg/l (NAA)	Mudoi et al. (2014)

B.nutans	Liquid MS+ 1 mg/l (BAP)	MS+ 1.0-5.0 mg/l (BAP)	½ MS +3.0 mg/l (NAA)/ 5.0 mg/l (IBA)	Islam and Rahman (2005)
B.nutans	MS+ 2.22 µM BA	Liquid MS+ 2.22 µM BA	MS+ 49.0 µM IBA	Yasodha et al. (2008)
B.nutans	MS+ 4.4 µM (BA)+ 2.32 (Kin)	Liquid MS + 13.2 µM (BA) + 2.32 µM Kin + 0.98 µM (IBA)	½ MS+ 9.8 µM (IBA) + 2.85 µM (IAA)+ 2.68 µM (NAA),	Negi and Saxena (2011)
B. oldhamii Munro	MS+0.45 µM (TDZ)+ 2.2 g/l Gelrite	Liquid MS + 2.27 µM (TDZ)	MS basal + 10.74- 26.85 µM (NAA)	Lin et al. (2007)
B.pallida	Liquid MS+ ascorbic acid 50 mg/L + citric acid 25 mg/L + cysteine 25 mg/L+ 1.34 µM (NAA)+ 1.125 µM (TDZ)	Liquid MS+ 1.34 µM (NAA)+ 4.44 µM (BAP)	½ MS+ 2% sucrose +1% glucose + 0.6% agar after treatment of butyric acid 0.5 mg/mL for 30 min	Beena and Rathore (2012)
Bambusapallida	MS+ 1 mg/l (BA)+ 2.5 % Gelrite	MS+3 mg/l (BA)	MS+ 2.0 mg/l (NAA)	Sharma and Sarma (2014)
B. salarkhanii	Liquid MS+ 1 mg/l (BAP)	MS+ 1.0-5.0 mg/l (BAP)	½ MS+3 mg/l (NAA)/5 mg/l (IBA)	Islam and Rahman (2005)
B. tulda	MS+1.0 mg/l (BA)	Semi-solid MS+1.0 mg/l (BA)	MS+5 mg/l NAA	Sharma and Sarma (2013)
B. tulda	semi-solid MS+ 10 µM BA + 0.1 µM IAA	MS (L) + 100 µM glutamine + 0.1 µM IAA+ 12 µM BAP	MS liquid medium + 40 µM coumarin	Mishra et al. (2008)
B. tulda	MS+ 3mg/L (BAP)	Liquid MS+ 2mg/L (Kn) + 3mg/L (BAP)	½ MS+ 3mg/L (IBA)+ 10mg/L coumarin+ 3% sucrose,	Waikhom and Louis (2014)
B. tulda	Liquid MS +2.5 mg/l BAP + 1mg/l Kn + 8% coconut water	Liquid MS+ 2.0 mg /l (BAP) + 1.0 mg/l Kin+ 8% coconut water	½ MS + 0.2 mg/l IBA	Das and Pal (2005b)
B. ventricosa	MS+ 22.2 µM (6-BA)	MS+ 22.2 µM (6-BA)+0.23 µM TDZ+ 0.27 µM NAA	MS + 2.7 µM NAA+ 4.9 µM IBA+ 4.4 µM 6-BA	Wei et al. (2015)
B.vulgaris	Modified MS+ 2 mg/l (BAP)	Modified MS+ 2 mg/l (BAP)	Modified MS+ 20 mg/l (IBA)	Ndiaye et al. (2006)
B. vulgaris varstriata	Liquid MS+ 1 mg/l (BAP)	MS+ 1.0-5.0 mg/l (BAP)	½ MS +3.0 mg/l (NAA)/ 5.0 mg/l (IBA)	Islam and Rahman (2005)
B. vulgaris	Liquid MS+ 1 mg/l (BAP)	MS+ 1.0-5.0 mg/l (BAP)	½ MS+3 mg/l (NAA)/5 mg/l (IBA)	Islam and Rahman (2005)
B.vulgaris	MS+ BAP (2.0 mg/ l)	MS +BAP (4.0 mg/l)	½ MS+ IBA (3.0 mg/l)	Ramanayake et al. (2006)
B. wamin	MS (L) + 5.0 mg/l (BAP)	Semisolid MS+ 2.0 mg/l (BAP) + 0.8 mg/ l (Kin)	½ MS+ 7.5 mg/l (IBA)	Arshad et al.(2005)
D. asper	MS+ 5 mg/l (BAP)	MS (L) + 5 mg/l BAP + 40 mg/l Ads	MS (L) + 1 mg/l (IBA)	Banerjee et al. (2011)
D.asper	MS+ 0.1-15 mg/l (BA)	MS (L) + 3 mg/l (BA)	MS + 10 mg/l (IBA)	Arya et al. (2002)
D.asper	MS + 15 µM BAP	MS + 10 µM BAP + 75 µM Ads+ 3% table sugar	½ MS + 5 µM IBA + 5 µM NAA	Singh et al.2013
D. asper	MS + 8.86 µM (BAP) + 13.5 µM (Ads)	MS+ 8.86 µM (BAP) + 13.5 µM Ads	MS+ 14.76 µM (IBA)+ 3.67 µM (NAA)	Nadha et al. (2013)

D.asper {Schult. &Schult. F.} Backer ex k. Heyne)	MS+15 µM BAP	MS + 10 µM (BAP)+ 75 µM Ads	½ MS + 5 µM IBA + 5 µM NAA	Singh et al.(2012b)
D.asper	MS+ 3 mg/l BA	IMS (L) + 3 mg/l BA + 50 mg/l Ads	MS (L) + 1.0 mg/l IBA	Kumar and Banerjee (2014)
D.BrandisiiKurz	MS (L) + Ascorbic acid (25mg/l) + Citric acid(12.5mg/l)+Cysteine-(12.5mg/l) + Glutamic acid-(50mg/l) + 0.25 mg/l TDZ+ 0.25 mg/l (NAA)	MS (L) + NAA (0.25mg/l) + BAP (2.5 mg/l)	½ MS (L)+ 1mg/l (NAA)	Kavitha and Kiran (2014)
D. giganteus Munro	Semi-solid MS+ 2 mg/l (BAP) + 0.1 mg/l (Kin)+ 1gm/l Benlate	MS (L) + 6 mg/l (BAP)+ 1 mg/l (kin) + 8% (v/v) coconut water	½ MS+ 3 mg/l IBA + 10 mg/l Coumarin.	Ramanayake and Yakandawala (1997)
D.hamiltoniiNees et Arn. Ex Munro	MS+ 2% sugar followed by MS+ 8 µM (BAP)+ 1µM (NAA)	MS+ 8 µM (BAP)+ 1µM (NAA)	MS+100 µM IBA followed by growth regulator free media	Agnihotri and Nandi (2009)
D.hamiltonii ARN. EX MUNRO	MS+ 3.0 µM TDZ	MS+ 1.5 µM TDZ + 56.0 µM ascorbic acid	½ MS+ 25.0 µM IBA + 36.0 µM choline chloride	Singh et al.(2012a)
D. Longispathus KURZ	MS+ 12 µM (BAP)+ 3µM (Kin)	MS (L) + 15µM (BAP)+ 1 µM (IBA) + 10% coconut water	½ MS+ 1 µM (IBA)+ 1 µM (IAA)+ 68 µM Coumarin.	Saxena and Bhojwani (1993)
D.strictusnees	White medium	Liquid MS+ 0.5 mg/l (BA)+ 0.5 mg/l (Kin)+ 200 ml/l coconut Water	Solid MS+0.25 mg/l IBA	Ravikumar et al.(1998)
D.strictusNees	MS (L)+ 0.5 mg/l (BA)+ 15 mg/l Ads	MS (L)+ 0.5 mg/l (BA)+ 15 mg/l Ads	1/2MS (L)+ 0.25 mg/l (IBA)	Chowdhury et al.(2004)
D. strictusNees	MS + 2 mg/l BAP	MS + 4 mg/l (BAP) + 15 mg/l (AdS)	MS+ 5mg/l (IBA)	Pandey and Singh (2012)
D.strictusNees	MS+ 0.5 mg/l (IAA)+ 15 mg/l Ads	----- -----	½ MS+1 mg/l (IBA)+ 1 mg/l (NAA)+ 0.5 mg/l 2,4-D+ 1 mg/l Phloroglucinol	Chaturvedi, et al.(1993)
D.strictusNees	MS+ 4 mg/ l (BAP)+ 0.25 mg/l (TDZ)	MS+ 4mg/ l (BAP)+ 0.25 mg/ l (TDZ)	Liquid MS+2.5mg/ l (BAP)+ 5 mg/ l (IAA),	Kapruwan et al. (2014)
D.strictus	MS+ 4 mg/l BAP	MS + 4 mg/l (BAP)	MS+ 3 mg/l (NAA)	Goyal et al.(2015)
Melocannabaccifera	MS+ 3mg/L (BAP)	Liquid MS+ 2mg/L (Kn) + 3mg/L (BAP)	½ MS+ 3mg/L (IBA)+ 10mg/L coumarin+ 3% sucrose,	Waikhom and Louis (2014)
Thamnocalamusspathiflorus (Trin.) Munro	½ strength MS	MS medium + 5.0 µM BAP + 1.0 µM IBA.	½ MS+ 150 µM IBA	Bag et al. (2000)
Thyrsostachysoliveri	Liquid MS+ 1 mg/l (BAP)	MS+ 1.0-5.0 mg/l (BAP)	½ MS +3.0 mg/l (NAA)/ 5.0 mg/l (IBA)	Islam and Rahman (2005)
BA- 6- Benzyl Adenine, BAP- 6- Benzyl aminopurine, TDZ- Thidiazuron, IBA- Indole -3-butyric acid, IAA- Indole -3-acetic acid, NAA- α-Naphthalene acetic Acid, Ads- Adenine sulphate.				

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