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# Regeneration Technique of Bamboo Species through Nodal Segments: A Review

Meena Maiya Suwal, Janardan Lamichhane, Dhurva Prasad Gauchan
Department of Biotechnology, School of Science, Kathmandu University, Dhulikhel, Kavre, Nepal.
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## Abstract
Micropropagation is an alternative technique to propagate at large scale plants to meet global plant demand. Various researchers have worked on the micropropagation technique to regenerate bamboo species by using nodal segments from years. Contamination, browning, necrosis, and acclimatization with physiological stress are the extreme problems of the micropropagation technique. But, many numbers of papers have been published on micropropagation of the bamboo species through nodal segments as explants. The proliferation of the bamboo shoots is dependent on the season of collection, size of explants, the position of explants, diversity of plants, concentration and combination of plant growth regulators, most adequate culture medium, environmental condition of the equipment, handling, and individual species. Bamboo is a monocarpic fast-growing, tall perennial grass and having the high potential to generate economic and social benefits. It helps to maintain land patterns and control soil erosion. The long life cycle of the bamboo produces a huge amount of seeds but unfortunately, mostly, they are non-viable. So, bamboos are propagated from vegetative by cutting and air layering. However, these methods are only for a small scale and they also tend to destroy large mother plant stocks and difficult to be transported. So, the *in vitro* propagation technique is useful to obtain large progenies from desired genotypes. Mostly, BAP and TDZ growth hormones are widely used for shoot multiplication and IBA, NAA and IAA are used for root initiation as per developed protocols in tissue culture for large scale production. This review intends to explore an overview of the recent literature reports to summarize the importance of micropropagation by using nodal segments of bamboo species and factors influencing it.

**Keywords**: Micropropagation, Nodal segments, Bamboo species, Plant Growth Regulators, Shooting and Rooting

Corresponding author, email: gauchan@ku.edu.np

## Introduction
Bamboo is an attractive plant species for various purposes due to its versatile utilization with high economic potentiality and its accelerated growing capacity at short period [1, 2]. It is perennial strong woody giant grass [1-5] having a unique complex branching system of rhizomes with root. It belongs to subfamily Bambusoideae of the Poaceae family and subsequently divided into three tribes [3, 4, 5, 6]. It can grow from approximately 75 to 400mm per day [7]. It is distributed up to 4000 m. a. s.l. in temperate to the zone of all continents except Europe [8]. It prefers to grow at a minimum of 100 cm annual rainfall with high atmospheric humidity in steep hillsides, road embankments, gullies, or on the banks of ponds and streams [9]. It occupies 3% of the forest in the tropical, subtropical, and temperate zone of the world but while, in Asia; it covers 10 % of the forest. There are 120 genera and 1641 species found in the world [10, 11 and 12]. Tropical Asia has rich bamboo diversity with up to 60 genera and 1000 species [13]. However, Nepal has only 12 genera and 65 species of bamboos distributed in the tropical, subtropical, and temperate zone [14].

Since the ancient period, bamboo species are widely used as a renewable source due to its versatile nature [15, 16]. According to Hsiung (1988), more than 4000 traditional uses and 1500 commercial applications have been known to bamboo. It's multipurpose potentiality from cradles to coffin and as "Green Gold" [17]. Similarly, it is used for making paper, pulp, food, scaffolding, textiles, plywood, boards, raw materials for construction, fencing, clothes, reinforcing fibers, etc, and bio-energy applications [10]. Also, it is used as an alternative source of energy and helped to prevent soil erosion due to the closely woven mat of intertwining roots and rhizomes [17, 18] with its high agro-climatic suitability.

It is perennial plants with monocarpic nature having vegetative and reproductive cycle range from 3 to 120 years [19], sporadic flowering with the recalcitrance of seeds which made difficult for identification and floral characterization of bamboo. Mostly, the classification and identification of bamboo is done based on the vegetative pattern but it is not reliable due to influence of ecological factors an easily practicable because bamboo can produce a limited number of seeds after a long period of its life span which has short viability period only 3 to 6 months [17, 18, 19] and suffering from insects and rodents. Hence, the bamboo is regenerated through the vegetative method by planting of rhizome off-set, branch & culm cuttings, nodal macro-cutting and layering [20, 21] but for this process, it needs a large mass of bamboo plant stocks. This leads to the destruction of entire clumps and the gradual depletion of bamboo resources. Again, it is not easily available sufficiently due to seasonal dependency and low rooting capacity so it is highly expensive [17]. Similarly, it is difficult to transport and handle due to heavyweight and long length [22, 23]. Also, there is more chance of liable desiccation before rooting [24]. Moreover, yield production of bamboo is hardly possible [22-27] through conventional and non-conventional methods. Therefore, it is necessary to apply other techniques of plant tissue culture because it is highly demanded the industry to agriculture.

## *In Vitro* Propagation of Bamboo

Plant tissue culture is the only advanced technique that can be applied to solve the challenges of speedy and mass propagation of the bamboo species [27]. For the conservation of bamboo and to fulfill the growing demand of the markets, micropropagation is the alternative method that provides rapid mass multiplication of bamboo along with disease-free plants as well as the same clone [25-32]. Micropropagation is not only ensuring the supply of quality planting material regularly but it also helps in conserving of germplasm of bamboo [25].

Different types of explants viz. seed, seedlings, inflorescences, root, culm, mature clumps, nodal segment, meristem domes or leaves, etc [33, 34, 35] are used for bamboo micropropagation. In bamboo, both juvenile and mature plants can be considered as explants [36]. The nodal segments containing is considered as more effective explants for *in vitro* culture because of resumed food materials. Due to the presence of highly active meristematic tissue in nodal segments, it develops into new plantlets [37]. The response of explants depends on the physical condition of plants, the health of mother plants, collection of the season from field and size of explants, and its position in mother plants [38]. Using nodal explants for organogenesis reduces the chance of somaclonal variation [27].

There is so much research conducted on micropropagation of bamboo through nodal explants [27-47] However, the developed protocols are either insufficient or not applicable because the protocols are only limited to the research which is not applicable for industrial mass production. It is intended to explore the suitable protocols on the micropropagation technique for mass bamboo propagation. This review intense to give an overview of the recent literature reports to summarizes the importance of micropropagation by using nodal segments of bamboo species and factors that influence it.

## Collection of explants

Nodal explants were collected from January to February, March to April, May to June, July to August), September to October, and November to December [32, 41, 48]. The establishment of explants in culture was directly related to the collection of explants seasons and plants species which influenced in pure culture. It was dependent on external factors i.e. contamination and concentration of the hormones in media [49]. Explants collected during February-March and September-October showed maximum bud break [48]. It was reported that the rainy season was better for bud break in *Dendrocalamus strictus* [49] *Bambusa tulda* [40] *Gigantochloa atroviolacea* [50] with the high rate of contamination [40, 51, 52]. The explants collected from November to January (winter months) produced only 35-45% bud break response during the culture of *Bambusa vulagaris* by [53]. Spring and summer season (February to June) was a comparatively better period for the collection of explants [37]. However, Negi and Saxena [29, 30] have obtained high aseptic cultures along with a 90% bud response from July to October in *Bambusa balcooa*. According to Negi and Saxena (2011) and Mehta et al. (2011), the best collection of explants was in July for culture initiation in *Bambusa nutans*. But Singh et al. (2012b) reported the collection of nodal explants at pre-monsoon induced maximum

bud break in *Dendrocalamus asper*. Ramanayake et al. (1995) observe that the influence of seasons on bud break was countable in *D. giganteus* and *Berberis vulgaris*. It was observed that seasonal effects on bud initiation and found that February to March is a good period for obtaining auxiliary buds for cultural development [54]. Moreover, Shivabalan et al. (2014) have established a new culture form explants of *B. balcooa* collected in December. In other seasons, the establishment of pure culture is difficult due to the high scale of contamination suffering from the variable pathogen in the summer and rainy season. Better establishment of pure aseptic cultures and bud response of explants depends on the ratio of contamination, handling, physiological state of explants, species, type of explants, and the season of collection [24, 43, 52, 55, 56, 57, 58, 59].

Different aged of the mother stock plants were also used for culture initiation. The new Culm (1 year) and lateral branched (frequently actively branched) of 2 to 5 years old bamboo can be used as explants [25, 43]. But Patel et al. (2015) have established *in vitro* culture from 2 to 30 years old explants and also Sharma et al. (2012) used nodal explants from the current year's growth mature and healthy clumps of *Bambusa nutans*. Similarly, nodal explants of 40 years old *B. nutans*; 30 years old *B. balcooa* [60] and 10 years old *B. tulda* were used for multi proposed [59, 61]. The aged of the explants could not effectually determine in the initiation of the shoot *in vitro*. Furthermore, there may be some result influenced by the age of the plants during the experiment but there was no impact reason behind it. It depended on the composition of media, concentration of hormones, contamination rate, and condition of the culture [31]. Also, various aged of the explants can be established successfully *in vitro* culture through single nodal segments from bamboos.

## Position of the Nodal Explants

To date, only limited researchers have mentioned the position of nodal explants in mother plants. Nodal segments from the healthy mature mother plant with disinfected lateral branches and were more effective for the initiation of the culture [62]. Chowdhury et al. (2004) recorded that the 1st and 2nd position from the base of secondary branches of *D. strictus* was the best for regeneration in micropropagation. But, according to Mudoi et al. (2008, 2014), the 5th to 7th position of the *B. balcooa*, *B. nutans* and *B.tulda* explants from mother stock culm was best for maximum regeneration *in vitro* culture rather than below 5th position because the base explants can excaudate phenolic compound which resulted in browning problem on shoots. A similar result was also illustrated in the report of Devi and Sharma (2009) in which the top position of explants showed a low frequency of bud break in comparison to the basal and mid Culm nodes in *Arrundinaria callosa* Munro. Middle node explants of Culm were very effective resulted *in vitro* propagation of the *B. vulgaris*, [63]. Another experiment revealed that the auxiliary branch of explants from healthy mother stock was found to be good for regeneration of the new plants such as in *D. hamiltonii* [1, 64], *D. asper* [65], *D. giganteus* [56]. Similarly, Sharma and Sarma (2013) reported that young lateral buds also showed the bud break in *B. tulda*. Therefore, it is stated that the top and the base portion of the nodal segment in Culm bamboo can hardly regenerate *in vitro* propagation of bamboo.

## Surface Sterilization

The size of 2.5 mm explants was more effective than a smaller size (5-7 mm) to initiate the culture within a short period because of high endogenous hormonal effect [37, 41]. For the initiation of the culture, the explants were surface sterilized by treating different kinds of chemicals for a certain time to avoid the contamination. It was reported that explants treated in 70% ethanol for the 30s to 1 min [25, 29, 30, 31, 33 39] followed running tap water after washing in 4-6 drops of detergent (Tween 20/Tween 80) for 30 mins. [29, 30, 33, 68] reduced the rate of contamination. It was reported that 0.1% Mercuric chloride ($HgCl_2$) was found more effective than other surface sterilants (Sodium hypochlorite, Potassium Hypochlorite, Hydrogen Peroxide, etc.) for various species of bamboo micropropagation [29-,31, 33, 35, 36, 38, 39, 65- 71]. So it is suggested that 0.1% Mercuric Chloride was more effective for the disinfection of the explants because the high concentration of the $HgCl_2$ retarded the growth of plants due to the impact of chemical in the internal tissues [72]. Wei et al. (2015) have reported that the treatment of explants on 0.1% $HgCl_2$ at lower duration enhanced the survival rate of explants and frequency of bud break. When treatment time was increased at the same concentration of $HgCl_2$ in *D. strictus,* the bacterial and fungal contamination was decreased [36]. Similarly, for the establishment of a pure culture of bamboo, different researchers have

used antiseptics like savlon, tempol, streptomycin sulphate, gentamicin, cetavelon, etc [29, 40, 42, 60] and fungicide like bavistin, benomyl, mancozeb, carbendazim, tetracycline, etc [37, 41, 42. 49, 53, 56].

## Culture Media

According to Chang and Ho (1997), the nutritional composition is greatly varied in culture media which depends on the types of tissues and plant species. So, for the establishment of the culture, the proper media play a major role in the growth and development of the plants. It is difficult to consider a unique media for all types of plants in tissue culture. Not only for getting maximum auxiliary bud breaking, but MS [78] medium has also been widely practiced for more superior responsive bud proliferation and further multiplication of bamboo in comparison to other 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) [34-122]. But Kabade (2009) observed that the WPM media is suitable for shoot induction from nodal segments of *B. bambos*. Similarly, Shirgurkar et al. (1996), Singh et al. (2001), Ogita et al. (2009), and Negi and Saxena (2011) reported that the half-strength rather than full strength MS medium was better for successful *in vitro* culture in bamboo [123-124].

The physical condition of the media is also a factor that influences to grow plant tissue under *in vitro* culture. Several researchers reported that the proliferation and shoot multiplication of the bamboo was successfully obtained under *in vitro* culture on semi-solid/solid MS media [34, 35, 36, 39, 40-48]. Mostly, 0.8% agar was widely used as a gelling agent to solidified/semi-solidified the media which influenced the plant metabolism [32-39, 76]. Some researchers also used phytagel (0.2%) or Gelrite (0.2-0.35%) to agar which influenced high bud breaking in *B. wamin* [72] and shoot proliferation in *B. oldhamii* [77]. Similarly, it was noted that dwarf and a lower number of shoots per explant in MS solid media due to leaching and browning problems [79]. Similarly, Sharma and Sarma (1998) have also observed leaching of phenolic exudates and poor growth of shoot in MS agar gelled medium. Several reporters mentioned that liquid MS media was also observed more suitable than MS semi-solid/ solid media for proliferation and multiplication of the shoots in bamboo species [34, 43, 78]. The high rate of shoot initiation has observed in the liquid medium compared to agar gelled medium means attributed to easy availability and faster uptake of nutrients in liquid medium [81]. When culture initiated in liquid media generally shoots were grown faster and less required hardening time [82].

## Plant Growth Regulators

The chemical substances which influenced either promote (positive) or inhibit (negative) the growth of the plant are plant growth regulators (PGR). The low quantity of PGR can change the morphological structure of plants. Natural and synthetics phytohormones are widely used in tissue culture. Mostly cytokinins and auxin are used for callogenesis and histogenesis of bamboo. Different concentration of the 6–Benzyl aminopurine (BAP), 6–Benzyl adenine (BA), Napthalene Acetic Acid (NAA), Indole 3-Butyric Acid (IBA), Indole Acetic Acid (IAA), Zeatin (ZN), kinetin (KN), Thidiazurn (TDZ) with the supplement of 3% sucrose and 100 mg/L Myo-Inositol was used on the micropropagation of the bamboo. The growth regulators hormones used by researchers in **Table 1**. There were various factors affect the initiation of an aseptic culture of explants. The rate of percentage on bud break was varied with different concentrations of plant growth hormones, condition, physiological status of explants, size, the position of the explants, age of mother plants, the health of mother stock and collection season of explants. Mostly, BAP and BA were widely used for micropropagation of bamboo because it might be cost-effective and autoclave nature [36], and ultimately BAP and BA showed successful results when in cooperated within MS media for bud breaking, proliferation, and multiplication of shoots of several bamboo species. But Arya et al. (2003) could not obtain an axillary bud break in *B. tulda* in the presence of BAP only. Venkatachalam et al. (2015) have reported that 85% bud break was obtained separately or a combination of different concentrations of BAP, NAA, and KN with a supplement of Additivesincooperation with MS solid media. Moreover, similar combined effects of two cytokinins (BAP and KN) in different concentrations have found successfully result in bud breaking and shoot initiation of *B. arundinancea* Retz. Wild [84]. However, In shoot initiation experiment, different researchers have tried auxin (NAA, IAA) along with different combinations of cytokinins (BAP, KN, and TDZ and also suggested that increased levels of BAP and KN retarded in bud initiation [23, 57, 58, 62, 78].

**Table 1.** Micropropagation from Nodal segments/Explants

| Bamboo Species | Plant Growth regulators | | | Results | Ref |
|---|---|---|---|---|---|
| | Bud breaking | Shoot Multiplication | Rooting | | |
| *Arundinaria callosa* Munro | Liquid MS + BAP(13.3 µM/L) | Liquid MS + BAP (13.3 µM/L) + IBA (1.0 µM/L) | 1/2 MS Liquoid + IBA ( 25 µM) + BAP ( 0.05 µM/L) | Shoot multiplication and Rooting | 70 |
| *B. arundinacea* | BAP (5.0 mg/L) | BAP (5.0 mg/L) | NAA (3.0 mg/L) | Mass multiplication | 79 |
| *B. arundinacea* Retz. Willd | MS + BAP(3.0 mg/L ) +KN (0.5 mg/L) | MS +BAP (3.0 mg/L) + KN (0.5 mg/L) | ½ MS+ IBA ( 2.0 mg/L) + KN (0.5 mg/L) | Mass multiplication | 94 |
| *B. balcooa* | - | BAP (11.25µM/L) +KN (4.5 µM/L) | 1/2 MS+ IBA (1.0 µM/L) | *In Vitro* regeneration | 67 |
| *B. balcooa* | BAP (1.0 mg/L) | BAP (1.0-5.0 mg/L) | 1/2MS+NAA(1-3mg/L) + IBA(1 -5. mg/L) | Mass multiplication | 112 |
| *B. balcooa* | MS +BAP (4.4 µM/L)+NAA (0.53µM/L) | MS + BAP (4.4 µM/L)+ NAA (0.53µM/L) | MS + NAA (16.11 µM/L) | Mass multiplication and Rooting | 49 |
| *B. balcooa* Roxb | MS+ citirc acid (25mg/L) + ascorbic (50 mg/L) +BAP (3.5 mg/L) | MS+ BAP (3 mg/L)+ NAA (0.5 mg/L) | MS +NAA ( 4 mg/L) | Mass multiplication and Rooting | 51 |
| *B. balcooa* | MS+TDZ (0.1 mg/L)+ Gelrite (2g/l) | MS + TDZ (0.1 mg/L) | MS + TDZ ( 0.01 mg/L) + 2,4-D (0.5 mg/L). | Mass multiplication and Rooting | 85 |
| *B.balcooa* | MS + BAP (1 mg/L) | MS+ BAP (1 mg/L) | MS+ BAP (1 mg/L) + NAA (3mg/L) | Mass multiplication and Rooting | 61 |
| *B.balcooa* | MS+ BAP (4 mg/L) | Liquid MS + BAP (4 mg/L) | MS Liquid+ IBA (1mg/L) | Mass multiplication and Rooting | 68 |
| *B.balcooa* | Liquid MS + BAP (1 mg/L) | MS+BAP (1.0-5.0 mg/L) | ½ MS+ NAA (3 mg/L)/ IBA (5 mg/L) | Mass multiplication and Rooting | 112 |
| *B.balcooa* | Liqiud MS + BAP (11.25 µM/L) + KN (4.5 µM/L) | Liquid MS + IBA (1 µM/L) | ½ MS Liquid) + IBA (1 µM/L) | Mass multiplication and Rooting | 98 |
| *B.balcooa* | MS+ BAP (4.4 µM/L) + KN (2.32 µM/L)+ Gelrite (0.2% w/v) | Liquid MS + BAP (6.6 µm/L)+KN ( 2.32 µM/L)+ Coconut water (2.5% (v/v) | 1/2 MS +IAA (5.71 µM/L)+ IBA (4.9 µM/L)+NAA (5.37 µM/L) | Mass multiplication and Rooting | 34, 35 |
| *B.balcooa* | MS + BAP (3 mg/L) | MS + BAP (5 mg/L) | MS +NAA (4.5 mg/L) | Mass multiplication and Rooting | 124 |
| *B. bambos* | MS + BAP (4.4 µM/L) | MS+ BAP (4.4 µM/L) + KN (1.16 µM/L) | MS+ IBA (9.80 µM/L) | Mass multiplication and Rooting | 49 |
| *B.bambos* | MS + BAP (4.4 µM/L)+KN ( 1.16µM/L) | MS + BAP (4.4 µM/L)+KN ( 1.16 µM/L) | MS + IBA (9.80µM/L) | Mass multiplication and Rooting | 49 |
| *B.bambos* | … | BAP (5.0 mg/L) | NAA (3.0 mg/L) | Micropropagat-ion | 48 |
| *B. edulis* | BAP (1mg/L)/ BAP(1mg/L)+ NAA(1mg/L) | TDZ (0.01 mg/L) | TDZ (0.01 mg/L) | Micropropag-ation and In- vitro flowering | 85 |
| *B.glaucescens*Willd | MS + BA (5 µM/L) | Liquid MS+ BA(5 µM/L) + KN 15 µM/L) | MS+ IBA (25 µM/L) | Mass multiplication and Rooting | 99 |
| *B. nutans* Wall ex. Munro | MS+ BAP (1.0 mg/L) | MS +BAP ( 0.5 mg/L) + NAA (0.1 mg/L). | MS+ NAA (2.0 mg/L) | Mass multiplication and Rooting | 29 |
| *B. nutans* Wall ex. Munro | Liquid MS+ BAP (1 mg/L) | MS+ BAP (1.0-5.0 mg/L) | ½ MS +NAA(3.0 mg/L)/ IBA (5.0 mg/L) | Mass multiplication and Rooting | 112 |
| *B. nutans* Wall ex. Munro | MS+ BA (2.22 µM/L) | Liquid MS+ BA (2.22 µM/L) | MS+ IBA (49.0 µM/L) | Mass multiplication and Rooting | 97 |
| *B.nutans* | MS + BAP (4.44 µM/L)+ 2,4-D (4.2 µM/L) + 3% Sucrose | MS + TDZ (6.49 µM/L)+ NAA ( 0.74 µM/L) | Ms +NAA (16.11 µM/L) +2% sucrose | Mass multiplication and Rooting | 58 |
| *B. nutans* Wall ex. Munro | MS+ BA (4.4 µM/L + KN (2.32) | Liquid MS + BA (13.2 µM/L) + KN (2.32 µM/L) + IBA (0.98 µM/L) | ½ MS+IBA (9.8 µM/L) + IAA (2.85 µM/L)+ AA (2.68 µM/L) | Mass multiplication and Rooting | 34, 35 |
| *B. nutans* Wall ex. Munro | MS + BAP (5.0 µM/L) | MS + BAP (5.0 µM/L) | MS + IBA (10.0 µM/L) | Shoot multiplication and Rooting | 119 |

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|---|---|---|---|---|---|
| *B. oldhamii* Munro | MS+ TDZ (0.45 µM/L)+ Gelrite (2.2 g/L) | Liquid MS + TDZ (2.27 µM/L) | MS basal + NAA (10.74-26.85 µM/L) | Shoot multiplication and Rooting | 85 |
| *B. pallida* | Liquid MS+ ascorbic acid (50 mg/L) + citric acid (25 mg/L) + cysteine (25 mg/L)+ NAA 1.34 µM/L+ TDZ (1.125 µM/L) | Liquid MS+ NAA (1.34 µM/L)+ BAP ( 4.44 µM/L) | ½ MS+ 2% sucrose +1% glucose + 0.6% agar after treatment of IBA (0.5 µM/L) for 30 min | Mass multiplication and Rooting | 111 |
| *B. pallida* | MS+ BA (1 mg/L) + Gelrite (2.5% ) | MS+ BA (3 mg/L) | MS+ NAA ( 2.0 mg/L) | Mass multiplication and Rooting | 88 |
| *B. salarkhanii* | Liquid MS+ BAP (1 mg/L) | MS+ BAP (1.0-5.0 mg/L) | ½ MS+ NAA (3 mg/L)/IBA(5 mg/L) | Mass multiplication and Rooting | 112 |
| *B. tulda* | MS+ BA (1.0 mg/L) | Semi-solid MS+ BA (1.0 mg/L) | MS+ NAA(5 mg/L) | Mass multiplication and Rooting | 88 |
| *B. tulda* | semi-solid MS+ BA (10 µM/L) + IAA ( 0.1 µM/L) | MS (L) + glutamine (100 µM/L ) + IAA (0.1 µM/L) + BAP (12 µM/L) | MS liquid medium + 40 µM/L Coumarin | Mass multiplication and Rooting | 47 |
| *B. tulda* | MS+ BAP (3mg/L) | Liquid MS+ KN (2mg/L) + BAP (3mg/L) | ½ MS+ IBA (3mg/L)+ coumarin 10 mg/L + 3% sucrose | Mass multiplication and Rooting | 46 |
| *B. tulda* | MS (Liquid) + BAP (8.8 µM/L)+ KN(4.46 µM/L) + 2% Sucrose | MS (Liquid) + BAP(8.8µM/L)+KN ( 4.46 µM/L) + 2% Sucrose | MS (Liquid) + IBA (18.8 µM/L) + 2% Sucrose | Mass multiplication and Rooting | 122 |
| *B. tulda* | Liquid MS + BAP (2.5 mg/L) + KN (1mg/L) + 8% coconut water | Liquid MS+ BAP (2.0 mg /l) + KN (1.0 mg/L)+ 8% coconut water | ½ MS + IBA (0.2 mg/L) | Mass multiplication and Rooting | 98 |
| *B. ventricosa* | BAP (4.44 µM/L) | BAP (4.44µM/L) | NAA (5.4 µM/L) + BAP (0.44 µM/L) | *In Vitro* regeneration | 53 |
| *B. ventricosa* | MS+ BA (22.2 µM/L) | MS+ BA (22.2 µM/L) + TDZ (0.23 µM/L)+ NAA (0.27 µM/L) | MS + NAA(2.7 µM/l) +IBA( 4.9 µM/L)+ BA (4.4 µM/l) | Mass multiplication and Rooting | 78 |
| *B.vulgaris* | Modified MS+ BAP(2 mg/L) | Modified MS+ BAP (2 mg/L) | Modified MS+ IBA (20 mg/L) | Mass multiplication and Rooting | 113 |
| *B. vulgaris* | Liquid MS+ BAP (1 mg/L) | MS+ BAP (1.0-5.0 mg/L) | ½ MS +NAA (3.0 mg/L)/IBA ( 5.0 mg/L) | Mass multiplication and Rooting | 112 |
| *B. vulgaris* | Liquid MS+ BAP (1 mg/L) | MS+ BAP (1.0-5.0 mg/L) | ½ MS+ NAA (3 mg/L) / IBA(5 mg/L) | Mass multiplication and Rooting | 112 |
| *B. vulgaris* | MS+ BAP (2.0 mg/ l) | MS +BAP (4.0 mg/L) | ½ MS+ IBA (3.0 mg/L) | Mass multiplication and Rooting | 64 |
| *B. wamin* | MS (L) + BAP (5.0 mg/L) | Semisolid MS+ BAP (2.0 mg/L) +KN (0.8 mg/ l) | ½ MS+ IBA (7.5 mg/L) | 8 | 80 |
| *D. asper* | BAP ( 0-2.0 mg/L + CW (0-20.0 mg/L) | BAP (5.0 Mg/L) | NAA (0.5 mg/L) | *In Vitro* culture | 2,96 |
| *D. asper* | MS+ BAP ( 5 mg/L) | MS (L) BAP ( 5 mg/L) +Ads ( 40 mg/L) | MS (liquid) + IBA (1 mg/L) | Shoot multiplication and Rooting | 103 |
| *D.asper* | MS+ BA (0.1-15 mg/L) | MS (L) + IBA (3 mg/L) | MS + IBA (10 mg/L) | Shoot multiplication and Rooting | 25 |
| *D. asper* | BAP (3.0 mg/L) | BAP (1.0-4.0 mg/L | NAA (2.0 mg/L) or IBA (10.0 mg/L) | Shoot multiplication and Rooting | 48 |
| *D.asper* | MS + BAP (15 µM/L) | MS + BAP (10 µM/L) + Ads (75 µM/L)+ Table Sugar 3% | ½ MS +IBA ( 5 µM/L) + NAA (5 µM/l) | Shoot multiplication and Rooting | 21 |
| *D. asper* | MS+BAP (8.86 µM/L)+ Ads (13.5 µM/L) | MS+BAP (8.86 µM/L) + Ads (13.5 µM/L) | MS+ IBA (14.76 µM/L)+ NAA (3.67 µM/L) | Shoot multiplication and Rooting | 59 |
| *D.asper* {Schult. & Schult.f.} Backer ex k. Heyne) | MS+ BAP (15 µM/L) | MS + BAP (10 µM/L)+ Ads (75 µM/L) | ½ MS + IBA (5 µM/l) + NAA (5 µM/L) | Shoot multiplication and Rooting | 66 |

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|---|---|---|---|---|---|
| *D.asper* | MS+ BA (3 mg/L) | Liquid MS + BA (3 mg/L) + Ads (50 mg/L) | MS (Liquid) + IBA (1.0 mg/L) | Shoot multiplication and Rooting | 2 |
| *D.Brandisii*Kurz. | MS (L) + Ascorbic acid (25mg/L) +Citric acid (12.5mg/L)+Cysteine (12.5mg/L) + Glutamic acid (50 mg/L) + TDZ (0.25 mg/L)+ NAA (0.25 mg/L) | MS (Liquid) + NAA (0.25 mg/L) + BAP (2.5 mg/L) | ½ MS (L)+ NAA (1mg/L) | Shoot multiplication and Rooting | 86 |
| *D. giganteus* | BAP (2.0 -5.0 mg/L) | KN (10.0 µM/L) + BAP (0.5 µM/L) | … | Mass multiplication | 73 |
| *D.giganteus* | BAP (30.0 µM/L) | BAP (20.0 µM/L) | IBA (25 µM/L) + BAP (0.05 µM/L) | Rapid multiplication | 48 |
| *D.giganteus* | .. | | IBA+TDZ+ Coumarin | Root induction | 105 |
| *D. giganteus* Munro | Semi-solid MS+ BAP (2 mg/L) + KN (0.1 mg/L)+ Benlate (1g/L) | MS (Liquid) + BAP(6 mg/L)+ KN (1 mg/L) + 8% (v/v) coconut water | ½ MS+ IBA (3 mg/L) + 10 mg/L Coumarin | Shoot multiplication and Rooting | 106 |
| *D.hamiltonii*Nees et Arn. Ex Munro | MS+ 2% sugar followed by MS+ BAP (8 µM/L)+ NAA (1 µM/L) | MS+ BAP (8 µM/L)+ NAA (1 µM/L) | MS+IBA (100 µM/L) followed by growth regulator free media | Shoot multiplication and Rooting | 102 |
| *D.hamiltonii* ARN. Ex MUNRO | MS+ TDZ (3.0 µM/L) | MS+ TDZ (1.5 µM/L) + ascorbic acid (56.0 µM/L) | ½ MS+ IBA (25.0 µM) + Choline Chloride (36.0 µM/L) | Shoot multiplication and Rooting | 62 |
| *D. Longispathus* KURZ. | MS+ BAP (12 µM/L)+ KN (3 µM/L) | MS (L) + BAP (15 µM/L)+ IBA (1 µM/L) + Coconut Water (10%) | ½ MS+ IBA (1µM/L)+ IAA (1µM/L) +Coumarin(68 µM/L). | Shoot multiplication and Rooting | 28 |
| *D. membranaceus* | MS +BAP (1-5mg/L NAA (0.5 g/L) | BAP (1-5mg/L) + NAA ( 0.5 mg/L) | NAA ( 3.0 mg/L) / IBA (10.0 mg/L) | Mass multiplication | 73, 79 |
| *D. membranaceus* | MS + BAP (4.4 µM/L) + KN ( 1.16 µM/L) | MS + BAP (4.4 µM)+ KN ( 1.16 µM/L) | 1/2 MS + NAA (5.37 µM/L) + BAP( 4.4 µM/L) | Mass multiplication and Rooting | 49 |
| *D. strictus* | MS + BAP (2.0-5.0 mg/L) | BAP (2.0-5.0 mg/L) | | Mass multiplication | 73 |
| *D. strictus* Nees | White medium | Liquid MS+ BA (0.5 mg/L)+ KN (0.5 mg/L)+ Coconut Water (200 ml/L) | Solid MS+ IBA (0.25 mg/L) | Shoot multiplication and Rooting | 1 |
| *D.strictus* Nees | MS (L)+ BA (0.5 mg/L)+ Ads (15 mg/L) | MS (L)+ IBA (0.5 mg/L)+ Ads (15 mg/L) | 1/2MS Liquid + IBA ( 0.25 mg/L) | Shoot multiplication and Rooting | 45 |
| *D.strictus* Nees | MS + BAP (2 mg/L) | MS + BAP (4 mg/L) + Ads (15 mg/L) | MS+IBA (5mg/L) | Shoot multiplication and Rooting | 75 |
| *D.strictus* Nees | MS+ IAA (0.5 mg/L)+ Ads (15 mg/L ) | | ½ MS+ IBA (1 mg/L)+NAA(1mg/L)+ 2,4-D (0.5mg/L)+ Phloroglucinol (1mg/L) | Shoot multiplication and Rooting | 56 |
| *D.strictus* Nees | MS+ BAP (4 mg/)+ TDZ (0.25 mg/L) | MS+ BAP (4mg/ L)+ TDZ (0.25 mg/ L) | Liquid MS+ BAP (2.5 mg/ L)+ IAA ( 5 mg/ L) | Shoot multiplication and Rooting | 31 |
| *D.strictus* Nees | MS+ BAP (4 mg/L) | MS + BAP (4 mg/L) | MS+ NAA (3 mg/L) | Shoot multiplication and Rooting | 44 |
| *D.strictus* (Roxb.) Nees | MS+ 2,4-D (5 mg/L) | ---------- | MS + 2,4-D (0.5 mg/L) | Callus initiation Shoot multiplication and Rooting | 31 |
| *Melocanna baccifera* | MS+ BAP (3 mg/L) | Liquid MS+ KN (2mg/L) + BAP (3mg/L) | ½ MS+ IBA (3 mg/L)+ Coumarin (10 mg/L)+ 3% sucrose, | Shoot multiplication and Rooting | 46 |
| *Thamnocalamusspathiflorus* (Trin.) Munro | ½ strength MS | MS medium + BAP ( 5.0 µM/L) + IBA (1.0 µM/L) | ½ MS+ IBA (150 µM/L) | Shoot multiplication and Rooting | 91 |
| *Thyrsostachysoliveri* | Liquid MS+BAP (1 mg/L) | MS+ BAP (1.0-5.0 mg/L) | ½ MS + NAA (3.0 mg/L)/ IBA (5.0 mg/L) | Shoot multiplication and Rooting | 112 |

## Contamination: Bottleneck for culture establishment

Surface and systemic contamination is the major incidence in tissue culture of bamboo because it has large intercellular spaces and vessel cavities at the cut end which accumulated contaminating agents deeply. Therefore, various bacterial and fungal contaminations have been serious problems obtained in bamboo *in vitro* culture. According to Ramirez et al. (2009), several bacteria like *Xanthomonas, Pseudomonas, Agrobacterium and Erwinia* and *Bacillus sp*. by Cruz- Martin et al. (2007) are contaminating agents in some species of bamboo tissue culture while *Pantoea agglomerans* and *P. ananatis* by Nadha et al. (2012) are the main bacterial contaminates in the nodal segments of *G. angistifolia*. To overcome this problem, there is either incorporation of antibiotics in media or explants treatment in antifungal. Surface sterilization of all pieces of equipment including explants has been done with care to escape contamination [35-40]. Yasodaha et al. (2008) have used Streptomycin and Kanamycin during the culture initiation stages. The combinations of Bavistin with streptomycin [55], streptomycin sulphate and tetracycline hydrochloride [68], bacteriocin [88] gentamycin [89] are used successfully to reduce contamination. When there is not directly used of tap water for washing explants during surface sterilization, it helps to minimize the contamination [38]. Bavistine (Cardazamine) and Mancozebas antifungal and Gentamycin as antibacterial [25, 41-45] are used as anti infections. Also, Ali et al. (2009) have been found that the combination of the antibiotics streptomycin, rifampicin and ciprofloxacin with the fungicide Bavistine is successfully disinfecting in the micropropagation of nodal explants of *B. tulda*, *B. balcooa*, *B. bamboos,* and *D. asper*. Oprin et al. (2004) found that rinsing explants with acetone for 3-4 times with a bleaching solution help to disinfect the explants. During surface sterilization of the explants, it is useful to apply the pre-treatment technique with the combination of the standard disinfected compounds. It is proved by Jimenez et al. (2006) when they pretreated the explants in Extran, Agri-mycine, and benomyl before surface sterilization in sodium hypochlorite and plant preservative mixture. The contamination was reduced from 2% to 11% in *Gaudua angustifolia*. Ramanayake et al. (1995) have suggested that it can be controlled systemic fungal contamination in *B. vulgaris* by supplement of benomyl in the culture medium.

Similarly, the other major problem for the bamboo *in vitro* propagation is browning and necrosis of the shoots in the initiation of culture, shooting stage and rooting stage due to phenolic compounds exudation [25] and increase the production of polyphenol oxidase by wounding of the tissue. It converts browning into blackish, and later on, it dies because the increased production of polyphenol is phytotoxic to the explants [45]. According to Huang et al. (2002) and Oprins et al. (2004), the browning of the plants depends on the species, age and position of the tissue, age of mother plants, the season of explants collection, used nutrient media and used sterilizing agents. Because of the exudation phenolic compound by the plant itself, there was encounter browning problem during shoots multiplication which decreased the multiplication rate. But Huang et al. (2002) observed that the browning of bamboo is in higher pH values of 7 and 8 while in the acidic nutrient media with standard pH 5.7 has a relatively low browning rate. Such a problem is solved by the supplement of some additives along with plant growth regulators in the media [46]. The browning is occurred according to species, tissue or organ, and nutrient medium *in vitro* [47]. Different types of antioxidants (Ascorbic acid, Cysteine, Activated Charcoal, Citric acid, Adenine sulphate, Polyvinylpyrrolidone (PVP) ) with various concentrations are either substituted into the media reduced the browning problem or soaking the explants in a liquid solution of those mentioned [73,74] to reduce the browning percentage in multiple shoots. Waikhom and Louis (2014) have shown that the addition of NaCl and Silicon in MS media significantly enhances the activities of antioxidant enzymes. But some researchers have succeeded to overcome serious browning and leaching problem by frequently transfer the clumps to the fresh medium without the incorporation of any antioxidants with media and treatments [74]. Huang et al. (2002) found in *B. oldhamii, D. latiflorus,* and *P. nigra* browning control when they used PVP, activated charcoal, ascorbic acid, cysteine, ferulic acid, and thiourea.

## Shoot Multiplication

The size and number of propagules have a vital role in the shoot multiplication. Three to four propagules for each culture to multiply was observed effective

[29-31] than individual propagule cultured [38]. Furthermore, BAP was extensively used on *in vitro* multiplication of different bamboos shoot [86, 87, 124]. A higher concentration of BAP was an impact on decreasing numbers and length of shoots. KN alone is not a significant response in shoot multiplication in *B. balcooa*, which results in the clumps dried and browning. Incorporation between KN and BAP was found effective in shoot multiplication [88]. Similar to the synergistic effect of KN and BA on the shoot multiplication rate was reported in *B. balcooa* [59]; *B. glaucescens* [89]; *D. giganteus* [40]; *B .tulda* and *M. baccifera* [38]. Similarly, TDZ has been reported an effective cytokinin for shoot proliferation [82, 90]. The effect of coconut water on bamboo shoot multiplication has been reported by different researchers. Saxena and Bhojwani (1993) have found that the addition of 10% coconut milk (CM) as an additive in the media is better for shoot proliferation and multiplication in *D. giganteus*. Ramanayake et al. (2001) reported that a high level of sucrose (4%) adversely affected the shoot multiplication in *D. giganteus*. There are 3% sucrose is widely used in tissue culture.

Devi and Sharma (2009) have found IBA was superior over NAA for shoot multiplication in *Arundinaria callosa* Munro. The use of IAA and NAA in conjunction with BA and KN was found to increase the length of shoots but lowered the multiplication rate [29]. Further, Gibberellic acid (GA) was effective and enhances for multiplication of shoot in *B. vulgaris* [63]. Rathore et al. (2009) only one researcher who has accounted for that the combined effect of NAA and BAP was effective for shoot multiplication of *B. balcooa and B. bambos*.

## Rooting

*In vitro* rooting is the bottleneck for the researchers in bamboo. Generally, NAA, IAA, and IBA are used individuals or combined for root initiation. These three hormones were more suitable for rooting in *D. asper* [84, 91,92,93,94, 97, and 98]. Clusters of 3-5 shoots were effective for transferring into rooting medium [29, 51, 58, 96, 99, 123, 124]. Full strength MS and half-strength MS media with the supplement of rooting hormones were frequently practiced for *In vitro* rooting. Arya et al. (2008) reported 80-90% of roots obtained in MS medium with supplementing of NAA or IBA within 5 weeks of transferring while working in *D. asper* and *D. falcatum*. But Singh et al. (2012b) have observed that 100% rooting in *D. asper* by using a combination of IBA and NAA. Whereas for *B. tulda* and *B. balcooa* a two-step treatment of 7 days on liquid MS medium supplemented with IBA and then transferred *in vitro* shoots to basal MS medium (pulse treatment) without any rooting hormones, was followed for *in vitro* rooting [43]. The combination of IBA and NAA for rooting has been also reported by Islam and Rahman (2005); Arya et al. (2006) and Rathore et al. (2009) in many important bamboo species. Some studies proved that IBA was found to be the most favorable root inducer compared to NAA and IAA on several bamboos such as *Drepanostachym falcatum* [40], *Oxytenanthera abyssinica* [94], *D. hookeri* [95], *D. hamiltonii* [92]); *Melocanna baccifera* [100]. On rooting medium shoots also elongated and good root and shoot system developed in 5-7 weeks. Saxena (1990) working on *B. tulda* has reported supplementing of Coumarin in rooting media resulted in better root induction and elongation. Similarly, Ramanayake and Yakandwala (1997) working on *D. giganteus* and Sood et al. (2009) on *D. hamiltonii* observed a high frequency of rooting when IBA was used in combination with Coumarin. In the case of *D. strictus*, up to 90%, rooting was found in medium containing IBA [38].

However, well-developed roots with healthy shoots were observed in half-strength MS medium supplemented with NAA [36]. Negi and Saxena (2011) have a document that the highest rooting frequency was obtained on ½ MS media with supplemented of IAA, IBA and NAA in *B. nutans* and also similar result obtained by Kapoor and Rao (2006) who reported that 100% rooting in ½ MS media containing the optimal concentration of BA and NAA in *B. bambos*. Sanjaya et al. (2005) have achieved *In vitro* rhizome in *P. stocksii* with continued subculturing of rooted plantlets on medium containing ½ strength of Major salts within the addition of IBA, BA, ascorbic acid, citric acid, cysteine and glutamine in different concentration. Chowdhury et al. (2004) obtained *in vitro* rhizome in *D. strictus* culturing in rooting media that have ½ strength major salts and IBA. Again, several researchers observed half-strength MS supplemented with NAA and IBA was better than that of the full strength of MS media [21, 25, 29, 78] in various bamboo species. Islam and Rehman (2005) have accounted that the couple of NAA and IBA

were suitable for the rooting of bamboo. But IBA was found better than NAA in *B. arundinacea* and *D. giganteus* for rooting by. Again, the IBA supplemented medium showed only poorly developed roots in *B.nutans* and *B. balcooa* [29]. But Ravikumar et al. (1998) reported that IBA supplement in MS media was more effective to root induction in *D. strictus* and *D. asper* respectively which was also supported by Bag et al. (2000) in the result of *Thamnocalamus spathiflorus*. Singh et al. (2012b) reported that the combination of IBA and NAA was a synergistic effect for rooting in *D. asper* in place of single use. But Mudoi and Borthakur (2009) observed a combination of NAA and BAP was found most effective in rooting of *B. balcooa* which was also confirmed by Goyal et al. (2015) in *D. strictus*. The limited number of the report was available for rooting in IAA. Kapruwan et al. (2014) reported IAA for *in vitro* rooting in *D. strictus*. The effect of growth regulators on rooting was varied species to species [95]. Negi and Saxena (2011) have practiced successfully rooting in full strength MS liquid media with the supplement of IAA, IBA, and NAA. And the high concentration of cytokine introduced for rooting might have resulted in cell death and cell cultures became yellowing leaves and reduced root mass in intact plants [97-100].

## Hardening

The transfer of *in vitro* propagated plantlets form Lab to land is another big nutshell of micro-propagation [22]. The plant developed *in vitro* is unable to survive *in vivo* directly due to lack of adaptation and proper hardening [43] however it has well-developed roots. To overcome the bottleneck of hardening, researchers have followed various hardening procedures. In general, the healthy and well-rooted plantlets are washed to free from the rooting medium and transferred to the pot containing growth supporting composition such as soil, sand, soil rite, perlite, cocopeat, agro peat, vermiculite, compost, farmyard manure, etc either alone or in various ratios [22]. Most researchers have used mention substrate in 1:1:1 ratio or modified. Some researchers have described the primary hardening and secondary hardening to obtained maximum numbers of plantlets. Like, *In vitro* plantlets were transferred to ½ strength MS liquid medium without plant growth regulators and vitamins for hardening in *D. asper* [65], *B. nutans* [109], and *D. hamiltonii* [54]. Then when plantlets transferred to polybags 1:1:1 composition of Sand: Farmyard manure: Soil they obtained high rate plantlets. The mortality percentage of plants is increased when the direct transfer of *in vitro* propagated plantlets to the external environment because of their inability to survive against biotic and abiotic stresses [91]. But Negi and Saxena (2011) obtained a 95.83% success rate by directly hardening in 2:1 mixture of soil: agro peat in *B. nutans*. Similarly, several workers reported on hardening of *in vitro* plants in the mixture of soil: sand: compost cocopeat (1:1:1) in *B.nutans* [87, 110]; soil: sand: cow dung(1:1:2) in *B. balcooa* [25] and *B. nutans* (Sharma and Sarma2014); soil mixture of peat, perlite, and vermiculite (1:1:1) in *B. oldhamii* [77], perlite, soil, and farmyard manure (1:1:1) in *D. strictus* [36]; 3:1 ratio of coco peat and vermicompost (3:1) in *B. balcooa* [43]. Without other substrates composition with soil, *In vitro* plantlet was acclimatized successfully in *B. bambos* [22, 29, 33, 41, 42, 62, 65]

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, KN- Kinetin, MS-Murrashige and Skoog media, CW-Coconut water.

## Conclusion

As a fast-growing and high potential economic development of the country, the demanding bamboo has enhanced the depleting rootstock of bamboo rapidly. Bamboo has a high capacity to carbon sequester and it is the mitigation of climate change and environments. Similarly, it is an alternative source of the forest. So that it has a great role in conservation biology and is become a priority concern. With knowledge of the awareness of the conservation biology and environment however people have to fulfill the enormous demands of the markets, they have to exploit the limited resources. Harvesting from the resources means that a large scale of bamboo plantlets are necessary highly through micropropagation to fill up the gap of plant stocks. Mass yield production protocols along with factors influence on it are discussed in the review. Several protocols are reported by several researchers. Nodal explants are better explants for micropropagation technique with the supplement of proper plant growth regulators in MS media at

decontamination condition, the proper season of explants collection, and appropriate position of the nodal segment in mother plant stocks. BA/ BAP is the best cytokine for bud initiation and shoots multiplication bamboo species. *In vitro* rooting is specific for bamboo species. IBA is a more effective rooting hormone for bamboo in comparison to other NAA and IAA. But several studies indicated that a couple of IBA and NAA is also effective for rooting in *in vitro*. It is also reported that the incorporation of NAA, IBA, and IAA is also suitable for rooting in some bamboo species. Sand alone is restricted for hardening but the combination of different substrates varies, the ratio is appreciable for hardening the *in vitro* rooted plants. The more relevant protocols have to develop by addressing those issues properly. Future research must be focused to generate a large scale of bamboo plants.

## Author's Contribution

MMS, JL and DPG were equally contributed to preparing framework of literature, conceptualization, Review and editing the final draft. MMS was involved in writing original draft of this review article. JL was Project coordinator and DPG was Project in charge. All authors read and approved the final manuscript.

## Competing Interest

The authors declare that they have no competing interest, which includes personal, financial, or any other kind of relationship with people or organizations that could inappropriately affect this review.

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### Abbreviations

Benzyl amino purine (BAP), 6 –Benzyl adenine (BA), Naphthalene Acetic acid (NAA), Indole 3-Butyric acid (IBA), Indole Acetic acid (IAA), Zeatin (ZN), Kinetin (KN), Thidiazurn (TDZ), MS (Murashige and Skoog), Plant Growth Regulators PGRs, Coconut Water (CW), Adenine Sulphate (Ads) etc.

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