

In vitro mass propagation of *Dendrocalamus asper* (Giant bamboo) through direct organogenesis

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Abstract

Dendrocalamus asper (giant bamboo) is a clumping type bamboo belonging to the family Poaceae. With its economic and environmental value, demand for this species has increased tremendously. Conventional propagation methods have limitations due to low seed viability and unavailability of healthy clumps. Therefore, an in vitro mass propagation protocol was developed to provide healthy plants for large scale plantations. Nodal segments were used for shoot initiation and the best medium for shoot induction, best medium for multiple shoot induction, effect of shoot cluster size and effect of physical state of the medium on multiple shoot induction were determined. Elongated shoots were transferred into rooting medium and best medium for root induction and effect of cluster size on rooting were determined. Well-developed plantlets were transferred to coir pellets and after four weeks transferred into different potting mixtures. According to the results, highest mean number of shoots per node (16.87 ± 0.52), mean shoot length (4.12 ± 0.27 cm) and mean number of leaves per shoot (4.80 ± 0.33) were observed in MS medium supplemented with 1.0 mg/L BAP. MS medium supplemented with 2.0 mg/L BAP was the best for multiple shoot induction, shoot clusters with 3 shoots was the best cluster size and liquid medium had better effect on shoot multiplication. Half strength MS medium supplemented with 2.0 mg/L IBA was the best for in vitro root induction with highest mean number of roots (7.15 ± 0.77) and mean root length (10.79 ± 1.11 cm). Shoot clusters with 3 shoots was the best cluster size for root induction. Sand: compost: coir dust (1:1:1) mixture was the best potting mixture giving 100% survival. These findings provide a reliable micropropagation protocol for *D. asper*, which holds great promise for meeting the growing demand for bamboo resources and promoting sustainable bamboo cultivation.

Key message

Findings of this research provide a reliable micropropagation protocol to regenerate healthy *D. asper* plants for large scale plantations to meet the growing demand for bamboo resources and promoting sustainable bamboo cultivation.

INTRODUCTION

Dendrocalamus asper is an evergreen clumping type bamboo belonging to the family Poaceae which is commonly known as giant bamboo, edible bamboo or sweet bamboo (Banerjee et al. 2011; Narin and Kanokporn 2021). The origins of *D. asper* are not definitive, but according to Benton 2015, they are distributed across India and Southeast Asia, including Thailand, Vietnam, Malaysia, Indonesia and the Philippines (Clark et al. 2015). The species was introduced to other tropical countries, including Kenya, Madagascar and Sri Lanka (Grubben 2008). Sri Lanka is a tropical country with distinct climatic zones and geographic areas that provide habitats for a wide array of bamboos. A report published in the "Bamboo/Rattan research Project in Sri Lanka" indicated that *D. asper* was initially established in the Royal Botanic Gardens, Peradeniya (Kariyawasam 1998) and then cultivated in the intermediate zone since 1862 (Dassanayake and Fosberg 1980), but the extent to which they are grown is not known as they are distributed as scattered small patches all over Sri Lanka.

D. asper is considered a multipurpose tree species. The primary use of this bamboo species is growing along the riverbanks against soil erosion and watershed rehabilitations (Wong 2004). Young shoots are edible; giving the species the general name “edible bamboo” (Singh et al. 2012). The sweet bamboo shoots are primarily used fresh, dried, shredded, pickled, canned or fermented (Choudhury et al. 2012). After three to four years of growth, they matured and are heavily used in the construction industry, especially as scaffolding or for making bridges. Due to its unique qualities in strength and durability (Awalluddin et al. 2017), the species has been introduced for diverse uses such as making furniture, musical instruments, household utensils, outriggers of fishing boats and paper making (Grubben 2008; Singh et al. 2012). Due to high levels of carbon sequestration, the species is considered as eco-friendly forest restoration species (Wang et al. 2013; Huang et al. 2014). Additionally, *D. asper* has the potential to be used as an ornamental plant in landscaping and interior decorations. The utility value of bamboo is expanding with the discovery of new applications (Ramanayake et al. 2007). In Sri Lanka, *D. asper* is mainly used in cottage handicraft industry, housing and construction purposes and in the construction of bridges and floor coverings (Kariyawasam 1998).

With the increasing demand for bamboo, the economic importance of bamboo resources has increased tremendously over the past couple of years. Even though the demand has increased, cultivation has remained restricted to abundant areas in the villages. Furthermore, some of the plants growing healthily along the waysides are ignored or indiscriminately eradicated due to unawareness on the species among the general public. Nevertheless, there are no established bamboo plantations in Sri Lanka. The only possible way to increase bamboo resources in the country is to establish large scale plantations.

Regeneration of *D. asper* through seeds and vegetative parts are the two commonly practiced conventional propagation methods. Propagation through seeds has limitations due to the unavailability of high-quality seeds because of irregularity in flowering and seed production. Some of the bamboo species are reported to have a flowering cycle of 150 years. Another drawback in using bamboo seeds for propagation is the short-term viability of seeds (Ayana et al. 2012; Mudoi et al. 2013). In general, the viability of seed decreases in a few weeks. However, even with viable seeds, the germination percentage is reported to be very low. Propagation through vegetative parts is generally carried out through rhizomes and culm cuttings which is always difficult due to the limited production of those parts (Pattanavibool 1998; Ramanayake et al. 2007). Thus, developing an efficient and reliable in vitro mass propagation protocol for *D. asper* is a current need in Sri Lanka and beyond.

MATERIALS AND METHODS

Seeds of *D. asper* that originated in Uttarkhand state of India were imported through a privet company. They were initially kept under running tap water for 15 minutes and treated with 2.0 g/L Captan for 5 minutes. After that they were treated with 5% Clorox mix with a few drops of Tween 20. Each step was followed by two successive washings with sterile distilled water. Then seeds were treated with 70% ethanol for 30 seconds followed by two successive washings with sterile distilled water. Finally, surface sterilized seeds were inoculated on growth regulator free MS medium for germination. The seedlings

obtained were maintained by subculture them regularly into fresh media for two months. Once the seedlings were established, they were used to obtain explants for in vitro direct shoot induction.

In vitro shoot induction

The nodal segments taken from in vitro germinated seedlings were cultured on MS medium supplemented with different concentrations of BAP (0.0–2.5 mg/L) for shoot initiation. The number of shoots per node, mean shoot length and mean number of leaves per shoot were recorded after 6 weeks of incubation.

Multiple shoot induction

Initiated shoot clusters were carefully separated into small shoot clusters and transferred into MS medium supplemented with increased concentrations of BAP (0.0–5.0 mg/L) for shoot multiplication and elongation. The mean number of shoots (> 0.5 cm), mean shoot length and mean number of leaves per shoot were recorded after 6 weeks of incubation. Shoots were multiplied for up to 12 culture cycles (5–6 weeks/cycle) without any loss of vigor in the multiplication medium.

Determination of factors affect shoot multiplication

Effect of shoot cluster size

Shoot clusters containing 1–4 shoots were cultured on MS medium supplemented with 2.0 mg/L BAP to determine the best cluster size for multiplication of shoots. Mean number of shoots (> 0.5 cm) per cluster, mean shoot lengths and mean number of leaves per shoot were recorded after 6 weeks of incubation.

Effect of physical state of the medium

Shoot clusters with 3 shoots were transferred to semisolid and liquid media separately. MS medium supplemented with 2.0 mg/L BAP and 8.0 g/L agar was considered the semisolid medium, while the same medium without agar considered as the liquid medium. The mean number of shoots (> 0.5 cm), mean shoot length and mean number of leaves per shoot were recorded after 6 weeks of incubation.

In vitro root induction

Elongated shoots (> 2 cm) were transferred into half-strength MS medium supplemented with different concentrations of IBA and IAA (0.0–5.0 mg/L). Cultures were incubated and observed weekly over a period of 6 weeks. Number of roots and root length (roots less than 1.0 cm were neglected) were measured by removing rooted plantlets from the vessel before being transferred for acclimatization.

Determination of factors affect root induction

Effect of shoot cluster size

Shoot clusters containing 1–4 shoots were cultured on half strength MS medium supplemented with 2.0 mg/L IBA. Mean number of roots (> 1 cm) and mean root lengths were recorded after 6 weeks of incubation.

Acclimatization

Well-developed and well-rooted plantlets were removed from the culture media without disturbing the roots and washed carefully with tap water to remove all traces of culture media. Then they were dipped in a fungicide solution (Captan 1.0 g/L) for five minutes and initially transferred to coir pellets (Jiffy products). Plantlets were maintained in a humid chamber and gradually exposed to ambient conditions. Percentage survival, shoot length and number of leaves were measured after four weeks. After four weeks of acclimatization in coir pellets, plantlets were transferred into different potting mixtures to determine the best potting mixture for tissue cultured plantlets. Albert's solution (2.0 g/L) was applied once a week as a liquid fertilizer and plants were watered regularly to maintain moisture levels. After eight weeks, survival percentage was calculated.

Culture media and growth room conditions

MS medium (Murashige and Skoog 1962) was used as the basal medium and the pH of the medium was adjusted to 5.7 ± 0.5 . The temperature of the growth room was maintained at $25 \pm 1^\circ\text{C}$ and light was provided by cool white fluorescent lighting to reach the 3,000 lx photosynthetically active radiation (PAR) under a 16 hour photoperiod. The humidity of the growth room was maintained at 50%.

Statistical analysis

A completely Randomized Design (CRD) was used in all experiments. Culture vessels were randomized at seven-day intervals and the means of obtained results were taken. Analysis of variance (ANOVA) was performed and pairwise comparisons were carried out using Tukey's test and least significant difference (LSD) at 5% confidence level. Growth regulator free MS medium was used as the control. Unless otherwise mentioned there were at least twenty replicates in all treatments.

RESULTS AND DISCUSSION

In vitro shoot induction

After 2 weeks of incubation, breaking of buds appeared on cultured nodal segments and shoots were induced from all the tested treatments (Fig. 1). According to the results (Table 1), after six weeks of incubation highest mean number of shoots per node (16.87 ± 0.52) and highest mean shoot length (4.12 ± 0.27 cm) were observed in nodes cultured on MS medium supplemented with 1.0 mg/L BAP. The mean number of leaves per shoot (4.80 ± 0.33) was also considerably higher in the same medium.

The lowest shoot formation was observed in BAP free medium (control), which produced mean number of shoots of 1.73 ± 0.3 with a mean shoot length of 2.63 ± 0.17 cm and mean number of leaves $6.00 \pm$

0.44. However, this indicates that even growth regulator free medium could be used for in vitro shoot induction but the success rate is very low.

Table 1
Effect of BAP concentration on in vitro shoot induction from nodal segments

BAP concentration (mg/L)	Mean number of shoots $\pm$ SE	Mean shoot length $\pm$ SE	Mean number of leaves $\pm$ SE
0.0 (Control)	1.73 $\pm$ 0.3	2.63 $\pm$ 0.17	6.00 $\pm$ 0.44
0.5	2.87 $\pm$ 0.19	2.61 $\pm$ 0.116	4.33 $\pm$ 0.23
1.0	16.87 $\pm$ 0.52	4.12 $\pm$ 0.27	4.80 $\pm$ 0.33
1.5	13.53 $\pm$ 0.68	1.29 $\pm$ 0.07	4.73 $\pm$ 0.37
2.0	6.93 $\pm$ 0.55	1.90 $\pm$ 0.31	2.47 $\pm$ 0.16
2.5	3.93 $\pm$ 0.41	1.74 $\pm$ 0.14	2.93 $\pm$ 0.3
LSD 5%	0.01	0.01	0.01

According to the study of Arya et al. 2008, 56.67% shoot initiation was observed when immature explants of *D. asper* were cultured on MS medium supplemented with 2.0 mg/L BAP. An increasing pattern of *D. asper* shoot formation (up to 30 shoots) and 2.8 cm maximum shoot length were observed with the increase in BAP in the medium (1.0 to 10.0 mg/L) after 6 weeks of incubation (Shroti et al. 2012).

Multiple shoot induction

After six weeks of incubation, shoots cultured on MS medium supplemented with 2.0 mg/L BAP showed the highest increase in mean shoot number (11.73 $\pm$ 1.59), and mean shoot length (9.21 $\pm$ 0.55 cm). The mean leaf number (12.2 $\pm$ 1.21) also considerably high compared to that in the other treatments (Table 2) (Fig. 2).

Table 2
Effect of BAP concentration on multiple shoot induction

BAP concentration (mg/L)	Increment of mean number of shoots $\pm$ SE	Increment of mean shoot length $\pm$ SE (cm)	Increment of mean number of leaves $\pm$ SE
0.0 (Control)	0.86 $\pm$ 0.18	7.29 $\pm$ 1.09	10.4 $\pm$ 0.75
1.0	4.40 $\pm$ 0.44	6.17 $\pm$ 1.07	12.47 $\pm$ 4.23
2.0	11.73 $\pm$ 1.59	9.21 $\pm$ 0.55	12.2 $\pm$ 1.21
3.0	8.33 $\pm$ 1.26	6.29 $\pm$ 0.88	9.47 $\pm$ 1.22
4.0	7.27 $\pm$ 2.27	3.99 $\pm$ 0.34	8.2 $\pm$ 1.22
5.0	6.13 $\pm$ 0.68	3.8 $\pm$ 0.56	8.06 $\pm$ 0.85
LSD 5%	0.01	0.01	0.01

There are several reports on use of the BAP for in vitro shoot multiplication in bamboos (Arya et al. 2008; Arya and Arya 2009; Shroti et al. 2012). According to the study of Arya et al. 2008, after 4 weeks of incubation, a mean number of shoots of 24.0 and mean shoot length of 2.2 ± 0.4 cm were observed in MS medium supplemented with 3.0 mg/L BAP. However, according to the study of Shroti et al. 2012 higher number of shoots (46.5), but less elongated (1.8 cm mean shoot length) shoots were observed in the presence of 3.0 mg/L BAP. Compared to the previous reports, in present study, at 2.0 mg/L BAP fewer but more elongated shoots with higher number of leaves were observed. More elongated shoots could be considered as a better character, although the multiplication rate was low compared to previous studies, as longer shoots are easy to root and establish in the soil for acclimatization.

Determination of factors affect shoot multiplication

Effect of shoot cluster size

After 6 weeks of incubation, a significant difference in shoot multiplication was observed with initial shoot cluster size. Cluster with 3 shoots was found to be the best cluster size (Fig. 3), with highest mean number of shoots per cluster (13.25 ± 0.61), an increase in mean shoot length (10.04 ± 0.35 cm) and mean number of leaves per shoot (15.88 ± 0.69). When a single shoot was introduced into MS medium supplemented with 2.0 mg/L BAP, the multiplication rate was very low and increased with cluster size (Fig. 4). However, it was observed that when the initial cluster is too crowded multiplication rate and the vigor decrease.

It was reported that a 3–4 propagule size of *D. asper* gave a 12 to 15 shoot multiplication rate with 3.0 mg/L BAP and lower concentrations of BAP reduced the multiplication rate while increased BAP levels (5.0–10.0 mg/L) produced large number of shoots (Arya et al. 2008). However, in the same study, low multiplication rate (3 to 6) was reported during the initial stages. According to Shroti et al. 2012 an

increasing pattern of mean no of shoots (6.54 to 48.74) was observed when propagule size increased from 1 to 5 on MS medium supplemented with 3.0 mg/L BAP and the highest multiplication rate (14.7) was observed with 3 shoot propagules. The results obtained in the present study also showed that an increased size of propagules reduced the multiplication rate and the rate of multiplication observed in the current study was higher than that in previous reports. The difference in the multiplication rate might be due to the differences in the origin of the explants used in this work.

Effect of physical state of the medium

After 6 weeks of incubation, shoot clusters cultured on liquid medium had a significantly higher mean number of shoots (21.3 ± 1.53), mean shoot length (12.15 ± 0.6 cm) and mean number of leaves per shoot (16.6 ± 1.9) than shoot clusters cultured on semisolid medium with a mean shoot number of 13.6 ± 1.36 , mean shoot length of 10.65 ± 0.45 cm and mean leaf number of 15.4 ± 1.7 (Fig. 5).

In vitro root induction

When shoots were cultured on half strength MS medium supplemented with different concentrations of IBA or IAA, there was a significant difference in the mean root length and number of roots per shoot. All tested treatments including growth regulator free MS medium (control) developed roots and half strength MS medium supplemented with 2.0 mg/L IBA was the best treatment for in vitro rooting (Fig. 6), with the highest mean number of roots (7.15 ± 0.77) and mean root length (10.79 ± 1.11 cm). The percentage root production ability in this treatment was 96%. From the half strength MS medium supplemented with IAA, medium with 3.0 mg/L IAA induced the highest mean number of roots (6.8 ± 0.5) with highest mean root length of 9.94 ± 0.72 cm and 92% root production.

Determination of factors affect root induction

Effect of shoot cluster size

According to the results, the mean number of roots and mean root length increased up to the cluster size of three shoots per propagule and decreased afterwards. Cluster with 3 shoots was found to be the best cluster size (Table 3) (Fig. 7), with highest mean number of roots per cluster (8.7 ± 0.64) and highest mean root length (11.25 ± 0.71 cm) resulting in a 100% rooting percentage. When single shoots were introduced into rooting medium, they showed the lowest mean number of roots per cluster (2.2 ± 0.53) and mean root length (4.8 ± 1.33 cm) resulting in only 15% rooting. *D. asper* is a sympodial type of bamboo and the rhizome is a major part of the plant that takes up and stores nutrients. It produces new shoots and roots for vegetative propagation of the plant thus, new root or shoot initiation depends on the amount of rhizome carried by each shoot cluster in vitro.

Table 3
Effect of initial shoot cluster size on in vitro root initiation

Shoot cluster size	Mean number of roots $\pm$ SE	Mean root length $\pm$ SE (cm)	Percentage of rooting %
1	2.2 $\pm$ 0.53	4.8 $\pm$ 1.33	15
2	3.45 $\pm$ 0.6	6.78 $\pm$ 1.22	55
3	8.7 $\pm$ 0.64	11.25 $\pm$ 0.71	100
4	6.05 $\pm$ 0.51	9.94 $\pm$ 1.12	100
LSD 5%	0.10	0.11	

Acclimatization

During acclimatization, micropropagated *D. asper* plantlets were successfully established in coir pellets and 100% survival was observed. When transferred to different potting mixtures, sand: compost: coir dust (1:1:1) potting mixture was found to be the best with 100% survival rate (Fig. 8), while other mixtures, exhibited lower survival rates due to water logging and root rot issues.

In vitro raised plantlets of *D. asper* have been acclimatized using different methods by different researchers. In their study, Arya et al. 2008 acclimatized in vitro raised *D. asper* plantlets in an open agro-net shade house in poly bags containing a mixture of sand:FYM:soil at a ratio of 1:1:1 for 2 months and eventually established in the soil with 80–90% survival. (Shrotri et al. 2012) successfully acclimatized *D. asper* plantlets in cocopeat under greenhouse conditions. In the present study, 100% survival was observed with healthy, vigorous growth. Large scale micropropagation can be successful only if plants show high survival rates after transfer from culture to soil and if the cost involved in the process is low.

CONCLUSIONS

According to the results mass propagation of *D. asper* is possible with in vitro micropropagation through direct organogenesis using nodal segments of in vitro germinated seedlings. The highest shoot formation was observed in nodal segments of in vitro germinated seeds in MS medium supplemented with 1.0 mg/L BAP. Six weeks old shoots can be used for shoot initiation on a large scale. MS medium supplemented with 2.0 mg/L BAP was the best medium for shoot elongation and multiplication. Propagules of 3 shoot clusters in liquid MS medium supplemented with 2.0 mg/L BAP increased the rate of shoot multiplication. Rooting of in vitro shoots was successful in half strength MS medium with either 2.0 mg/L IBA or 3.0 mg/L IAA with over 95% success. The above media compositions with propagules of 3 shoots increased the rate of root initiation. The use of coir pellets and maintaining plantlets in a humid chamber is recommended for a higher survival rate and sand: compost: coir dust 1:1:1 potting mixture was the best for maintaining plants in the nursery. Thus, the feasibility of large-scale cultivation and successful establishment of *D. asper* could be concluded.

Declarations

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AUTHOR CONTRIBUTION

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by PHC. The first draft of the manuscript was written by MDKMG and WTPSKS proof read the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by PHC. The first draft of the manuscript was written by MDKMG and WTPSKS proof read the manuscript. All authors read and approved the final manuscript.

Data availability statement

The authors declare that the data supporting the findings of this study are available within the paper. If any raw data files be needed in another format, they are available from the corresponding author upon reasonable request.

References

1. Arya ID, Arya S (2009) Propagation of bamboos through culture technology and field plantation. In: VIII WORLD BAMBOO CONGRESS. pp 131–143
2. Arya S, Satsangi R, Arya ID (2008) Direct regeneration of shoots from immature inflorescences in *Dendrocalamus asper* (edible bamboo) leading to mass propagation. *Bamboo Sci Cult* 21:
3. Awalluddin D, Ariffin MAM, Osman MH, et al (2017) Mechanical properties of different bamboo species. In: MATEC web of conferences. EDP Sciences, p 01024
4. Ayana DA, Tadesse Z, Kebede Y, Ayana DA (2012) Effect of storage media and storage time on germination and field emergence of *Oxytenanthera abyssinica* seeds. *Int J Basic Appl Sci* 1:218–

5. Banerjee M, Gantait S, Pramanik BR (2011) A two step method for accelerated mass propagation of *Dendrocalamus asper* and their evaluation in field. *Physiol Mol Biol Plants* 17:387–393
6. Benton A (2015) Priority species of bamboo. *Bamboo Plant Its Uses* 31–41
7. Choudhury D, Sahu JK, Sharma GD (2012) Bamboo shoot: microbiology, biochemistry and technology of fermentation-a review
8. Clark LG, Londoño X, Ruiz-Sanchez E (2015) Bamboo taxonomy and habitat. *Bamboo Plant Its Uses* 1–30
9. Classic Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15:473–497
10. Dassanayake MD, Fosberg FR (1980) A revised handbook to the flora of Ceylon. Taylor & Francis
11. Grubben GJH (2008) Plant Resources of Tropical Africa (PROTA). Prota
12. Huang Z, Li Y, Jiang P, et al (2014) Long-term intensive management increased carbon occluded in phytolith (PhytOC) in bamboo forest soils. *Sci Rep* 4:3602
13. Kariyawasam D (1998) Bamboo resources and utilization in Sri Lanka. *Bamboo Conserv Divers Ecogeogr Germplasm Resour Util Taxon*
14. Mudoi KD, Saikia SP, Goswami A, et al (2013) Micropropagation of important bamboos: a review. *Afr J Biotechnol* 12:
15. Narin C, Kanokporn P (2021) Effect of thickness on qualities of dried sweet bamboo shoots (*Dendrocalamus asper* Backer) products. *J Food Health Bioenvironmental Sci* 12:1–8
16. Pattanavibool R (1998) Bamboo research and development in Thailand. In: *Bamboo—conservation, diversity, ecogeography, germplasm, resource utilization and taxonomy*. Proceedings of a training course cum workshop. pp 10–17
17. Ramanayake S, Meemaduma VN, Weerawardene TE (2007) Genetic diversity and relationships between nine species of bamboo in Sri Lanka, using random amplified polymorphic DNA. *Plant Syst Evol* 269:55–61
18. Shrotri RK, Upadhyay R, Niratkar C, Singh M (2012) Micropropagation of *Dendrocalamus asper* through inter nodal segment. *Bull Environ Pharmacol Life Sci* 1:58–60
19. Singh SR, Dalal S, Singh R, et al (2012) Micropropagation of *Dendrocalamus asper* {Schult. & Schult. F.} Backer ex k. Heyne): an exotic edible bamboo. *J Plant Biochem Biotechnol* 21:220–228
20. Wang B, Wei WJ, Liu CJ, et al (2013) Biomass and carbon stock in Moso bamboo forests in subtropical China: characteristics and implications. *J Trop For Sci* 137–148
21. Wong KM (2004) Bamboo, the amazing grass. *Int Plant Genet Resour Inst IPGRI Univ Malaya Kuala Lumpur*

Figures

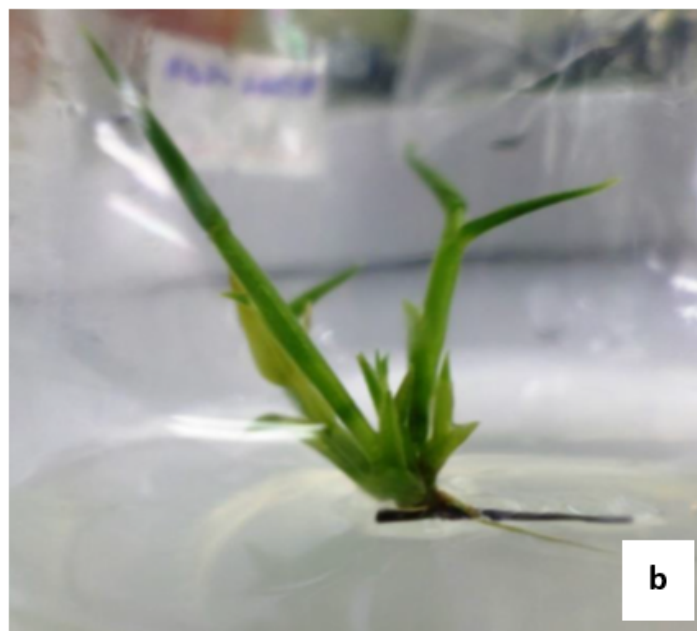
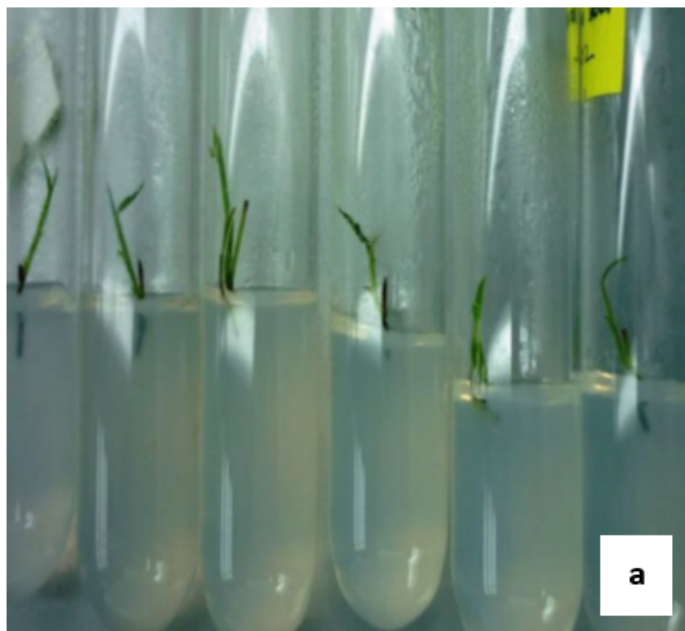


Figure 1

Shoot induction from nodal segments, (a) after two weeks of incubation, (b) after 4 weeks of incubation

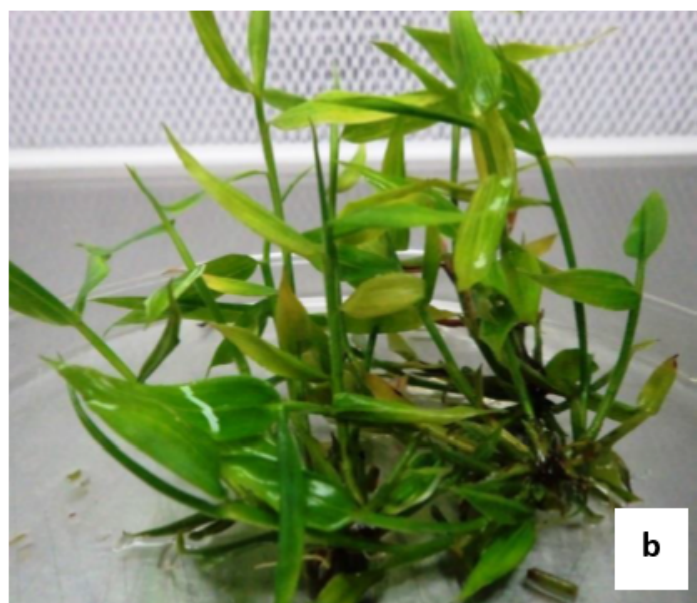


Figure 2

Multiple shoots induced in MS medium supplemented with 2.0 mg/L BAP

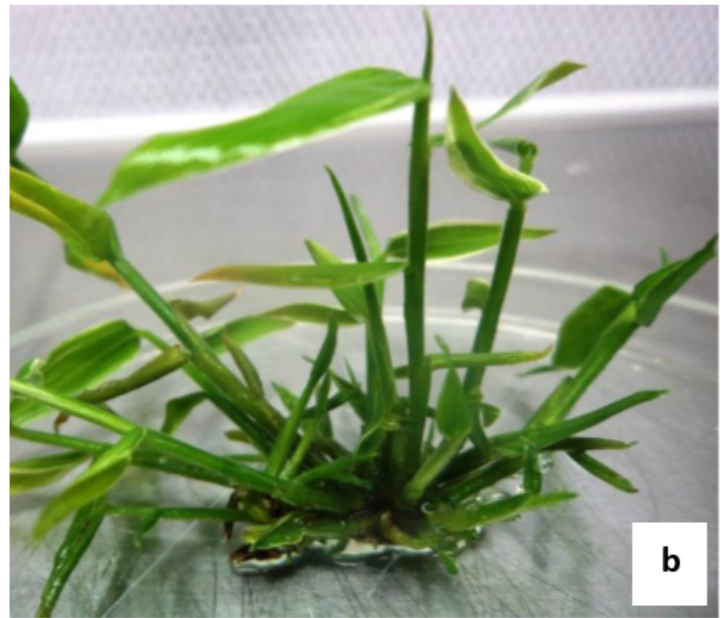
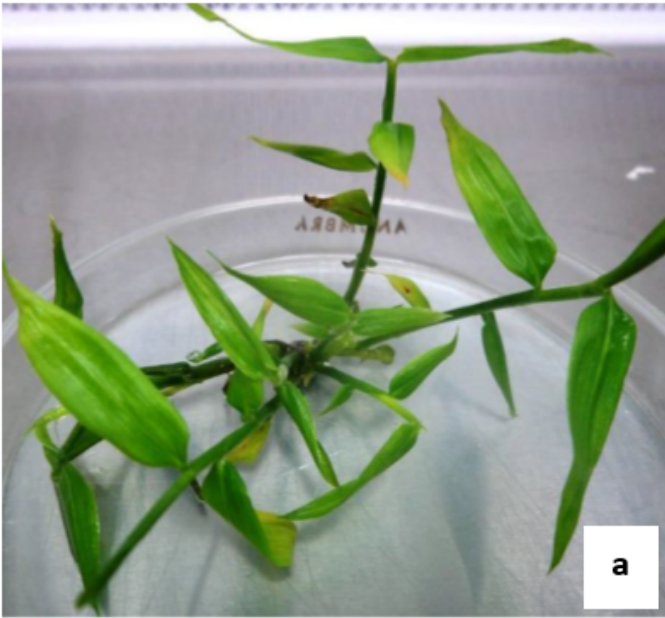


Figure 3

Shoot multiplication based on initial shoot cluster size (a) 2 shoots per cluster and (b) 3 shoots per cluster

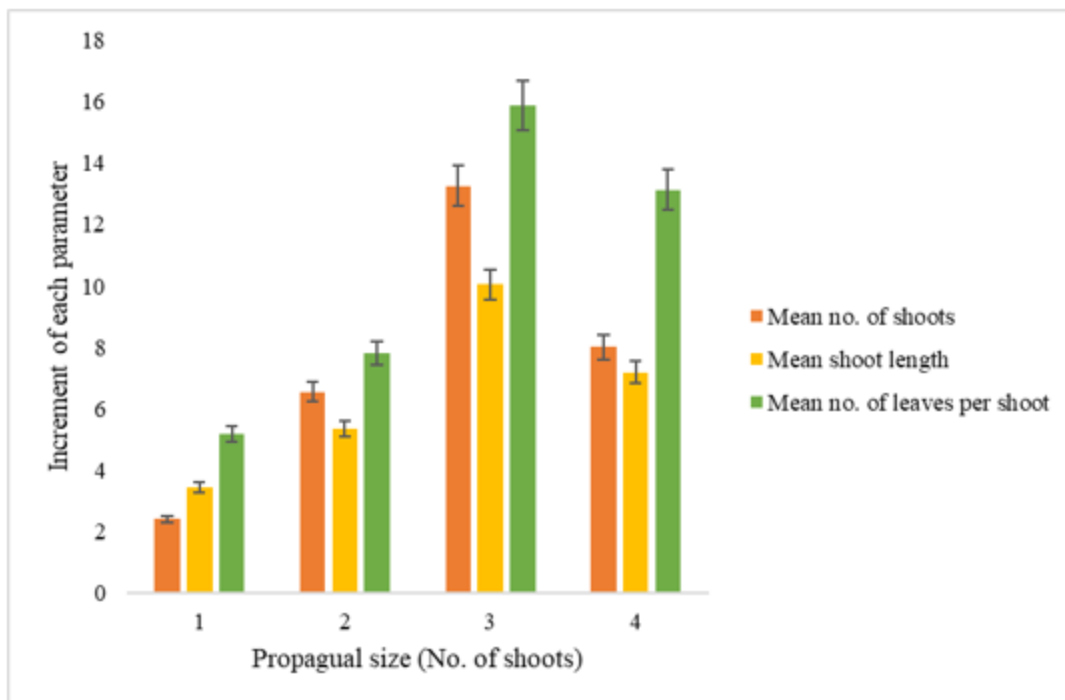


Figure 4

Effect of shoot cluster size on shoot multiplication

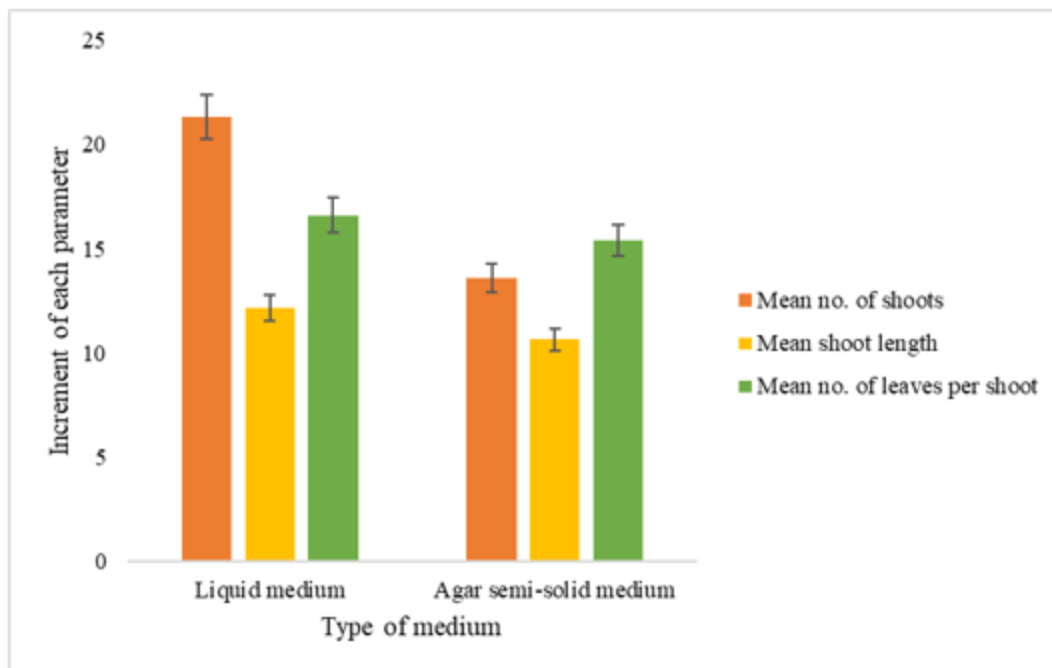


Figure 5

Effect of liquid and semi-solid medium on shoot multiplication

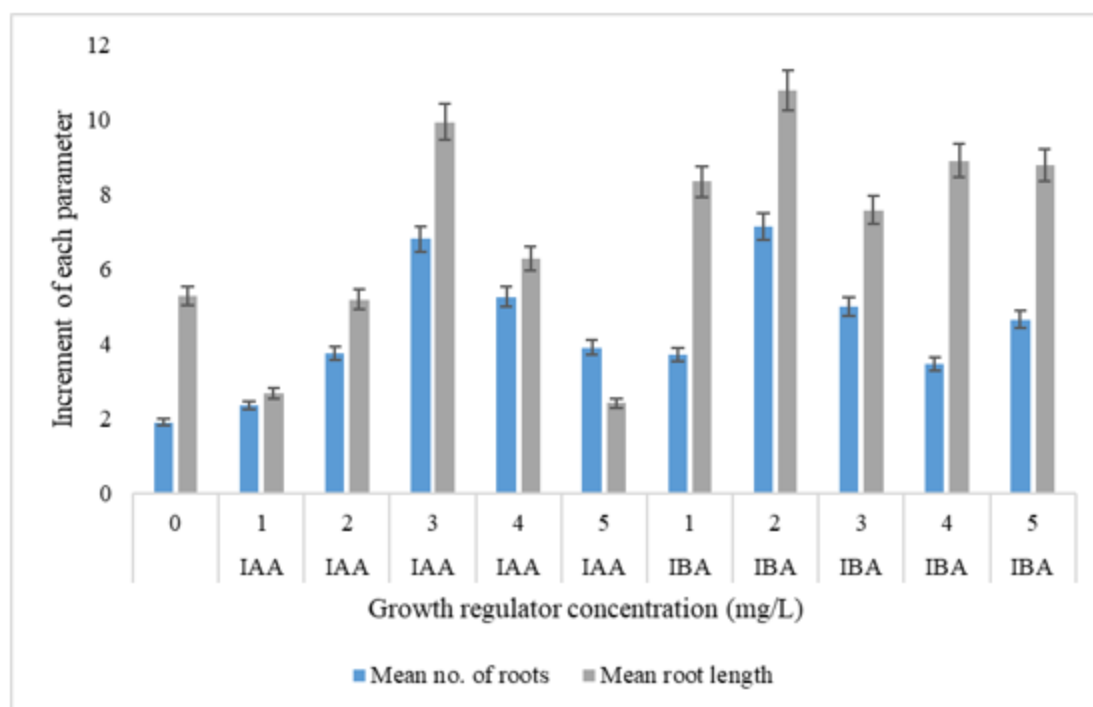


Figure 6

Effect of IAA and IBA concentrations on in vitro root induction

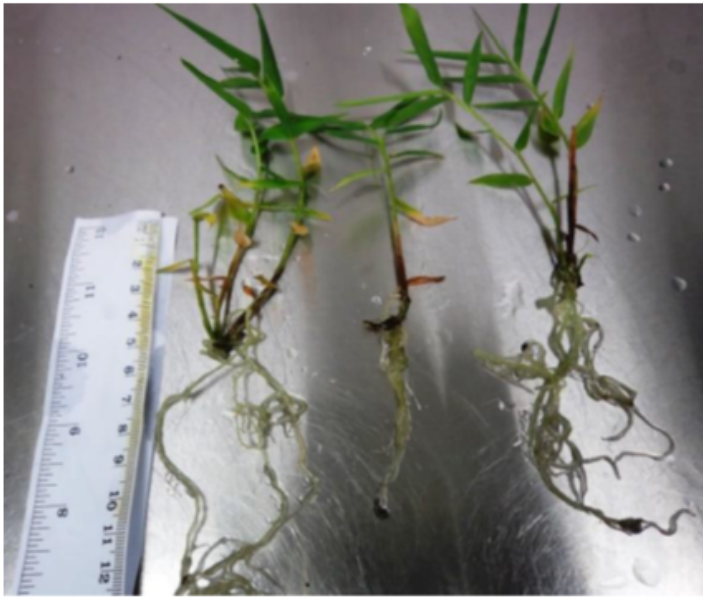


Figure 7

Variation of root formation with initial shoot cluster size



Figure 8

Acclimatization of in vitro raised *D. asper* plantlets in (a) coir pellets, (b) potting mixture containing 1:1:1 (Compost: sand: coir dust)