Botanica Orientalis – Journal of Plant Science (2019) **13**: 29–33 ISSN 1726-6858

Research article

Effects of cytokinins and triiodobenzoic acid on *in vitro* culture of *Bauhinia purpurea* L.

Belai Meeta Suwal Singh*

Patan Multiple Campus, Patan, Lalitpur, Nepal

Abstract

Bauhinia purpurea L. is a moderate-sized tree with multipurpose value. The main objective of this study was to evaluate the effects of different cytokinins on growth parameters and develop a standard protocol for nodes and shoot proliferations of *B. purpurea*. The cytokinins used in this study were 0.5 µM 6-benzylaminopurine (BAP), 5.0 µM 6-(4-Hydroxy-3-methyl-trans-2-butenylamino)-purine (Zeatin) and 5.0 µM N-benzyl-9(2-tetrahydropyranyl)-adenine (BPA), and these were combined with 1.0, 2.0 and 5.0 µM of 2,3,5-triiodobenzoic acid (TiBA). MS medium was used for the experimental purpose. Multiplication rate of plants was recorded after 8 weeks of culture. Best grown plants were acclimatized and stabilized well by natural rooting. Among the combinations, 1.0 µM TiBa and 5.0 µM BPA exhibited the best results in terms of multiplication of nodes, elongation of shoots and length and breadth of calli, thus this combinations can be used for propagation of *B. pupurea* for large scale multiplication.

Key-words: 2,3,5-triiodobenzoic acid (TiBA), 6-(4-hydroxy-3-methyl-trans-2-butenylamino)-purine (Zeatin), 6-benzylaminopurine (BAP), acclimatization, micro-propagation, N-benzyl-9(2-tetrahydropyranyl)-adenine (BPA), nodal explants.

Introduction

Bauhinia purpurea L. (Figure 1) is a moderate-sized tree belonging to the family Fabaceae (= Leguminosae), possessing ornamental, fodder and medicinal values. It is native to tropical South Asia, tropical and subtropical Himalaya and Myanmar. In Nepal, it has been planted in the plain and hilly region up to the elevation of 1600 m above sea level. The tree yields gum and the ethnobotanical reports made by Manandhar (2002) showed that fruits of *B. purpurea* are cooked and also pickled. The wood is used for making agricultural implements and is suitable for scanting and rafters in inferior construction work. *Bauhinia purpurea* is used in traditional medicines. Recent researches have highlighted that the plant possesses antibacterial, antidiabetic, analgesic, anti-inflammatory, anti-diarrheal, anticancerous, nephroprotective and thyroid hormone-regulating activities (reviewed in Kumar and Chandrashekar 2011). Pettit *et al*. (2006) isolated new and very remarkable cancer cell growth inhibitors (dibenzan L b, floxipens, designated as bauhiniastatins 1–4) from *Bauhinia purpurea*.

Micropropagation of *Bauhinia purpurea* has successfully been carried out by Kumar (1992) using Murashige and Skoog (MS) medium (Murashige and Skoog 1962) with 5.0 µM kinetin. Similarly, *in vitro* regeneration of *Bauhinia vahlii* has been developed by Dhar and Upreti (1999) using MS medium supplemented with 2.5 µM kinetin plus 100 mg/l adenine sulphate. Marthur and Mukunthakumar (1992) developed *in vitro* propagation protocols for two leguminous trees, *Bauhinia variegata* and *Parkinsonia aculeata* from nodal explants of mature tree using MS medium with 13.3 µM and 8.9 µM 6-benzylaminopurine (BAP) respectively. The aim of the present work is to evaluate the effects of cytokinins, BAP, 6–(4–hydroxyl–3–methyl-trans-2-butenylamino)–purine

*Corresponding email address: belaisingh@gmail.com

(Zeatin) (Zin), and n-benzyl–9(2–tetrahydropyranyl)-adenine (BPA), each supplemented with 2,3,5-triiodo-benzoic acid (TiBA) on growth parameters of *B. purpurea* and to develop a standard protocol for nodes and shoots proliferations, and the acclimatization of the best *in vitro* grown plants in the field.

Figure 1. Flowering branch of *Bauhinia purpurea*.

Materials and Methods

The seeds of *B. purpurea* were procured from district Afforestation Division Hattisar, Kathmandu, Nepal and were carried to the Institute of Pharmacognosy, Vienna, Austria and were preserved at 4˚C in refrigerator until experimental use. The healthy seeds were washed with few drops of Teepol detergent solution. They were soaked in distilled water for an hour prior to sterilization. The soaked seeds were washed with distilled water for 5 times and sterilized with 10% sodium hypochlorite solution for 10 minutes and removed the traces of sodium hypochlorite by washing thoroughly with sterilized distilled water five times inside a laminar air flow hood chamber. The seeds were again sterilized in 70% alcohol for one minute and washed with sterilized distilled water for 5 times to remove the alcohol. The seeds were inoculated on 8% (bacteriological) agar medium containing 3% sucrose, and the pH was adjusted to 5.8 before autoclaving. The seeds were sterilized at 15 lb./sq. inch pressure for 15 minutes in autoclave. Cultures were maintained at 25ºC (±2ºC). Cool white fluorescent light of an intensity of *ca*. 40 µ mol.$m^{-2}s^{-1}$ was supplied through OSRAM BIOLUX tubes at a 16 hr light period. Nodal explants obtained from germinated seedlings cultured on MS medium containing 0.5 µM BAP produced multiple shoots which were used for experimental purposes.

The nodal explants obtained from MS medium with 0.5 µM BAP were cut into 2 cm pieces and were placed on MS medium containing combinations of different cytokinins and TiBA under 4 different sets of experiments: (i) TiBA with concentrations of 1.0, 2.0 and 5.0 µM alone; (ii) 0.5 µM BAP in combination either with 0, 1.0, 2.0 and 5.0 µM TiBA; (iii) 5.0 µM Zin in combination either with 0, 1.0, 2.0 and 5.0 µM TiBA, and (iv) 5.0 µM BPA in combination either with 0, 1.0, 2.0 and 5.0 µM TiBA. The experiment was carried out in baby food jar of 200 ml capacity with 40 ml medium. In each of the vessel, 4 explants were inoculated. For the comparison, basal MS medium was used. The experiments were repeated in triplicate. The following measurements were made after 8 weeks of culture: number of nodes, shoot length elongations, and ϕ calli (length and breadth of calli in mm).

For acclimatization, the eight weeks old healthy plants, best grown *in vitro*, were removed from the culture and washed thoroughly in tap water to remove traces of nutrient medium and agar. Plastic pots (diameter 6 cm) were filled with soil (humus-ton substrate N8) and sand in 1:1 ratio and hardened in mist chamber. The substrate was disinfected by using Benlate and Previcure. The plants were kept at high humidity (80%) for two weeks; the humidity was reduced to (60%) and the acclimatization process continued for two weeks. The well rooted and acclimatized plants were transferred to green house for further hardening.

Results

Multiplications of nodal explants were not satisfactory in all concentrations of TiBA alone and calli were also not proliferated (Table 1). Nodal and shoot formations were satisfactory on MS medium with 0.5 µM BAP. MS medium supplemented with 1.0, 2.0 and 5.0 µM TiBA each with 0.5 µM BAP showed normal growth where the maximum number of nodes was 5.0 and shoot length was up to 33.95 mm and 6.8 mm ϕ calli were observed.

MS medium with 5.0 µM Zin showed good number of nodes (7.50) but shoot length proliferations were found only satisfactory (35 mm). In all the above mentioned concentrations of TiBA, each with 5.0 µM Zin, the node formations (range 3.75–4.52), shoot length proliferation (25.55–33.28) and ϕ calli (6.7–8.6 mm) were

recorded. Leaves were initiated in all the concentrations used.

MS medium with 5.0 µM BPA showed very interesting results producing 6.0 nodes and 52.90 mm shoot length proliferations with 18.75 mm ϕ calli. The lower concentrations of TiBA (1.0 and 2.0 µM) each with 5.0 µM BPA showed good multiplication of nodes (7.15–7.48), shoot length (43.37–57.47 mm), and proliferation of calli (11.65–12.35 mm ϕ; Figure 2). In higher concentrations of TiBA, the multiplication rate was declined. The calli formation decreased as TiBA concentrations were increased. The best proliferation was observed after 8 weeks of culture on MS medium with 1.0 µM TiBA with 5.0 µM BPA producing 7.48 nodes and 57.45 mm shoot length with 18.75 mm ϕ calli as compared to control containing only MS basal medium.

Figure 2. *Bauhinia purpurea* grown on MS medium with 1.0 µM TiBA and 5.0 µM BPA after 8 weeks of culture.

Table 1. Effects of various concentrations of TiBA with BAP, Zin and BPA on growth parameters of *Bauhinia purpurea*.

Additive/s in Media (µM)		Number of nodes/culture Mean ± SE	Shoot length (mm) Mean ± SE	ϕ Calli (mm) Mean ± SE
TiBA				
1.0		2.10 ± 0.1	11.95 ± 0.9	0.00 ± 0.0
2.0		2.20 ± 0.2	11.25 ± 0.5	0.00 ± 0.0
5.0		2.10 ± 0.1	9.20 ± 0.3	0.00 ± 0.0
TiBA	BAP			
0	0.5	5.75 ± 0.5	51.08 ± 6.7	8.70 ± 0.4
1.0		3.70 ± 0.3	25.63 ± 2.8	5.48 ± 0.4
2.0		5.00 ± 0.3	33.95 ± 2.9	6.80 ± 0.5
5.0		2.35 ± 0.2	15.35 ± 1.4	2.42 ± 0.3
TiBA	Zin			
0	5.0	7.50 ± 0.3	35.30 ± 2.1	10.70 ± 0.8
1.0		4.15 ± 0.3	33.28 ± 3.6	8.60 ± 0.4
2.0		3.75 ± 0.3	25.55 ± 2.0	7.15 ± 0.3
5.0		4.52 ± 0.3	25.73 ± 1.8	6.70 ± 0.3
TiBA	BPA			
0	5.0	6.00 ± 0.3	52.90 ± 5.3	18.75 ± 0.9
1.0		7.48 ± 0.4	57.45 ± 3.2	12.35 ± 0.3
2.0		7.15 ± 0.5	43.37 ± 2.3	11.65 ± 0.4
5.0		4.63 ± 0.4	26.12 ± 2.0	8.05 ± 0.5
Control		1.90 ±0.3	9.30 ±1.0	0.00 ±0.0

Discussion

In the present work, TiBA has an effect on reduction of multiplied nodes and shoot length elongation and no any proliferations of calli. Chen and Chang (2004) promoted the embryos of *Oncidium* on MS medium supplemented with 0.5 µM TiBA. MS medium supplemented with 0.5 µM BAP alone was found to be satisfactory for the growth of nodes as well as elongation of shoots. Effect of BAP alone and in combination with auxins on shoot proliferation has been worked out by other investigators. Kacer *et al.* (2005), for example, observed good multiplication of shoots (8.49) per explant on MS medium with 1 mg/l BAP in *Spathiphyllum* cv. 'Sweet Pablo'. Amatya and Rajbhandary (1993) induced multiplication of shoots of *Ficus semicordata* on MS medium with 0.5 mg/l BAP and 1.0 mg/l casein hydrolysate. Similarly, Dewan *et al*. (1992) produced multiple shoots of *Acacia nilotica* from cotyledonary node explants on Gamborg B_5 medium with 1.5 mg/l BA. Lakshmi (1981) induced shoots of *Eucalyptus citriodora* and *E. grandis* on MS medium supplemented with 0.5 mg/l BAP. In the present finding, the results obtained from the combinations of TiBA with BAP were not satisfactory, where only 2–5 nodes and 15–33 mm shoot elongations and 2–5 mm ϕ calli were recorded.

High concentration of Zin (5.0 µM) favored high rate of regeneration from nodes but the elongation of shoots was only moderate. But, Wawrosch *et al*. (2001) propagated *Lilium nepalensis* D. Don on MS medium supplemented with higher concentration (20 µM) of Zin using longitudinally split shoot halves. Maruyama and Ishii (1998) regenerated *Guazuma ulmifolia* Lam. from shoots taken from five-month-old potted seedlings on WPM containing 1.0 mg/l Zin. Similarly, San *et al.* (2013) found shoot multiplication of *Alnus glutinosa* (L) Gartn on WPM supplemented with 2.28 µM Zin. The results obtained from the combinations of TiBA and Zin were found optimum where only 3–4 nodes 25–33 mm shoots were observed but calli formations were found decreased as the concentration of TiBA increased. In a study, nodal explants of *Clerodendron phlomidis* L.F. cultured on MS medium supplemented with 9.12 µM Zin and 4 µM TiBA produced more and longer shoots than on medium without TiBA (Kher *et al*. 2016).

Among the combinations used in this study, MS medium with 5.0 µM BPA plus 1.0 µM TiBA exhibited the best results in terms of multiplication of nodes (number of nodes/callus being 7.48), elongation of shoots (shoot length being 57.45 mm) and ϕ calli (i.e., length and breadth of calli being 12.35 mm). These results were far better than the responses with BPA alone, in which 6 nodes, 52.90 mm shoots and 18.75 ϕ mm calli were produced. The number of nodes, shoot elongation, and calli formation were found decreased as the concentrations of TiBA increased. In conclusion, 1.0 µM TiBa and 5.0 µM BPA combinations can be used for propagation of *B. pupurea* for large scale multiplication.

Acknowledgements

Prof. Dr. Brigitte Kopp and Dr. Cristoph Wawrosch of the Institute of Pharmacognosy, University of Vienna, Austria; and Prof. Dr. Sanu Devi Joshi, Tribhuvan University, Kathmandu, Nepal are duly acknowledged for providing necessary supports. The Austrian Academic Exchange Service (ÖAD), Vienna, Austria is also acknowledged for financial support.

References

Amatya N. and Rajbhandary S.B. 1993. Tissue culture of *Ficus semicordata* for mass production. In: *National Conference on Biotechnology* (April 29–30), pp. 27. Nepal Botanical Society, Kathmandu, Nepal.

Chen J.T. and Chang W.C. 2004. TIBA affects the induction of direct somatic embryogenesis from leaf explants of *Oncidium*. *Plant Cell Tissue Organ Culture*, 79: 315–320.

Dewan A., Nanda K. and Gupta S.C. 1992. *In vitro* micro-propagation of *Acacia nilotica* subsp. *indica* Brenan. via cotyledonary nodes. *Plant Cell Reports*, 12: 18–21.

Dhar U. and Upreti J. 1999. *In vitro* regeneration of a leguminous liana (*Bauhinia vahlii* Wight and Arnott). *Plant Cell Reports*, 18: 664–669.

Kacer Y.A., Mazmanoglu M., Serce S. and Cetiner S. 2005. The effect of cytokinin type and concentration on multiplication rate of *Spathiphyllum* (Fam. Araceae). *Asian Journal of Plant sciences*, 4: 401–404.

Kher Mafatlal M., Sonar D., Shrivastava N., Natraj J.A. and Silva T. 2016. Micropropagation of *Clerodendron phlomidis* L.F. *Journal of Horticultural Research*, 24: 21–28.

Kumar A. 1992. Micropropagaation of a mature leguminous tree – *Bauhinia purpurea*. *Plant Tissue Culture and Organ Culture*, 31: 257–259.

Kumar T. and Chandrashekar K.S. 2011. *Bauhinia purpurea* Linn.: A review of its ethnobotany, phytochemical and pharmacological profile. *Research Journal of Medicinal Plants*, 5: 420–431.

Lakshmi S.G. 1981. Tissue culture of *Eucalyptus* species. In: *Tissue Culture of Economically Important Plants. Proceedings*

of International Symposium (A.N. Rao, ed.), pp. 180–182. COSTED and ANBS Publications, Singapore.

Manandhar N.P. 2002. *Plant and People of Nepal*. Timber Press, Oregon, USA.

Maruyama E. and Ishii K. 1998. Tissue culture of West Indian Elm (*Guazuma ulmifolia* Lam.), a multi-purpose tree in the tropical American region. In: *Challenges for Biotechnology in the Next Millenium*. *Proceedings of the 7th International Workshop of BIO-REFER*, pp. 139–144. BIO-REFER, Manila, Philippines.

Mathur J. and Mukunthakumar S. 1992. Micropropagation of *Bauhinia variegata* and *Parkinsonia aculeata* from nodal explants of mature trees. *Plant Cell Tissue and Organ Culture*, 28: 119–121.

Murashige T. and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, 15: 473–497.

Pettit G.R., Numata A., Jwamoto C., Usami M., Yamada T., Oshishi H. and Cragy G.M. 2006. Antineoplastic agents, isolation and structure of bauhinastatins 1–4 from *Bauhinia purpurea*. *Journal of Natural Products*, 69: 323–327.

San Jose M.C., Janeiro L.V. and Corredoira E. 2013. Micropropagation of threatened black alder. *Silva Fennica*. 47: 892. doi:10.14214/sf.892.

Wawrosch C., Malla P.R. and Kopp B. 2001. Clonal propagation of *Lilium nepalensis* D. Don, a threatened medicinal plant of Nepal. *Plant Cell Reports.* 20**:** 285–288.