European Journal of Medicinal Plants

31(10): 161-166, 2020; Article no.EJMP.58906
ISSN: 2231-0894, NLM ID: 101583475

Effects of Cytokinins on *in vitro* Culture of *Bauhinia purpurea* L.

Belai Meeta Suwal Singh[1*]

[1]Patan Multiple Campus, Patan, Lalitpur, Nepal.

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/EJMP/2020/v31i1030292
Editor(s):
(1) Dr. Daniela Rigano, University Federico II of Naples, Italy.
(2) Marcello Iriti, University of Milan, Italy.
Reviewers:
(1) Swati Kumari, ICAR – Central Institute for Subtropical Horticulture, India.
(2) Mehrcedeh Tafazoli, Sari Agricultural Sciences and Natural Resources University, Iran.
Complete Peer review History: http://www.sdiarticle4.com/review-history/58906

Original Research Article

Received 10 May 2020
Accepted 15 July 2020
Published 29 July 2020

ABSTRACT

Bauhinia purpurea L. is a moderate-sized tree with multipurpose value tree yields gum, its bark contains tannin and leaves are used as fodder. It is distributed in sub-Himalayan tracts. The main objective of this study was to evaluate the effects of different cytokinins on growth parameters of B. purpurea and develop a standard micropropagation protocol for nodes and shoot proliferations. The cytokinins used in this study were N-Benzyl-9(2-tetrahydropyranyl) (BPA), 6-furfurylaminopurine, Kinetin (Kn), 6-(4-Hydroxy-3-methyl-trans-2-butenylaminopurine) (Zeatin) (Zin), 2-isopentenyl aminopurine, (2-iP) and 6-benzylaminopurine (BAP) at four different concentrations (0.5, 1.0, 2.0 and 5.0 µM). Murashige and Skoog (1962) (MS) medium was used for the experimental purpose. Multiplication rate of plants was recorded after 8 weeks of culture. Such propagated best grown plants were acclimatized and transferred to the field. All the collected data were worked out statistically with SPSS, a system of analytical procedure.

Keywords: Micropropagation; cytokinin; nodal explants; acclimatization.

1. INTRODUCTION

Bauhinia purpurea L. (Fig. 1) is intermediate-sized tree belonging to the family Fabaceae (=Leguminosae), possessing ornamental and medicinal values. The common name of Bauhinia is Kachnar or white orchid tree. The bark of tree is rich in tannin and its leaves are used as

**Corresponding author: E-mail: belaisingh@gmail.com;*

fodder. The tree yields gum, used in traditional medicines and the ethnobotanical reports made by Manandhar [1] showed that fruits of B. purpurea are cooked and also pickled. The wood is used for making agricultural implements, is suitable for scanting and rafters in inferior construction work. 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. Recent researches have highlighted that the plant possesses antibacterial, antidiabetic, analgesic, anti-inflammatory, anti-diarrheal, anti-cancerous, nephroprotective and thyroid hormone regulating activities (Kumar and Chandrashekar, [2]. Pettit et al. [3] isolated new and very remarkable cancer cell growth inhibitors (dibenzan L b, floxipens, designated as bauhiniastatins 1–4) from B. purpurea.

Micropropagation of B. purpurea was successfully been carried out by Kumar [4] using Murashige and Skoog (MS) medium with 5.0 µM kinetin [4]. Similarly, in vitro regeneration of B. vahlii was developed by Dhar and Upreti [5] using MS medium supplemented with 2.5 µM kinetin plus 100 mg/l adenine sulphate]. Mathur and Mukunthakumar [6] developed *in vitro* propagation protocols for two leguminous trees, B. variegata and Parkinsonia aculeate from nodal explants of mature tree using MS medium with 13.3 µM and 8-9 µM BAP respectively.

The aim of present study is to evaluate the effects of cytokinins, N-Benzyl-9(2-tetrahydropyranyl) (BPA), 6-furfurylaminopurine, Kinetin, 6-(4-Hydroxy-3-methyl-trans-2-butenylaminopurine), (Zeatin) 2-isopentenyl aminopurine, 2-iP and 6-benzylaminopurine (BAP) on growth parameters of B. Purpurea to develop a standard protocol for nodes and shoots proliferations, and the acclimatization of the best in vitro grown plants in the field.

2. 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 4º til 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 to remove the traces of sodium hypochlorite residues it was further washed thoroughly with sterilized distilled water five times inside laminar air flow hood chamber. The seeds were sterilized in 70% alcohol for one minute and washed with sterilized distilled water for 5 times to remove the alcohol. The seeds were then dried with blotting paper and inoculated on 8% (bacteriological) agar medium containing 3% sucrose and the pH 5.8. 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 40 µ mol.m-2 s-1 was supplied through OSRAM BIOLUX tubes for a 16 hr light period. After 8-10 days, the nodal explants obtained from germinated seedlings were cultured on Murashig and Skoog's medium [7] containing 0.5µM BAP and produced multiple shoots which were used for experimental purposes.

The nodal explants obtained from MS medium with 0.5 µM BAP were cut into 5mm pieces and placed on MS medium supplemented with different cytokinins BPA, Kn, Zin, 2-iP and BAP at each of four different concentrations (0.5, 1.0, 2.0 and 5.0 µM) . The experiment was carried out in baby food jar of 200 ml capacity with 40 ml medium. In each of the culture vessel, 4 explants were inoculated. For the comparison, basal MS medium was used. The experiments were repeated in triplicate. All the results i.e. number of nodes, shoot length elongations, ф calli (length and breadth of calli in mm) were taken only after 8 weeks of culture.

Such cultured nodal explants were first subcultured in 0.5 µM BAP to multiply. The cultures were repeated till enough explants were obtained. For the large screening tests, common cytokinins such as BPA, Kin, Zin, 2-iP and BAP were added to the MS medium in the concentrations of 0.5, 1.0, 2.0 and 5.0 µM separately and compared with control basal medium. For statistical reliability each of the experiments were performed twice and the Mean ± SE (standard error) was calculated by SPSS, a system of analytical procedure.

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.

3. RESULTS

Nodal explants cultured on MS medium supplemented with 0.5, 1.0, 2.0 and 5.0 µM BPA, the best shoot length elongation was observed at the concentration of 5.0 µM BPA. The calli also increased 18.75 (Q) mm (length and breadth of calli mass) (Fig. 2).

Kinetin did not produce nodes as well as elongation of shoots or initiations of callus. On the other hand, 5.0 µM Kin produced only 3.15 nodes and shoot elongation of 31.30 mm with 8.25 Q (mm) calli. The nodal explants cultured on MS medium supplemented with 5.0 µM Zin showed the best results with maximum numbers of nodes (7.5) and elongation of shoots 35.50 mm with 10.70 Q (mm) calli. The plants produced were dark green, rigid and easy to propagate.

Fig. 1. Flowering plant of *B. purpurea*

Fig. 2. Plants growing on BPA 5.0 µM

Table 1. Effects of different cytokinins in *Bauhinia purpurea* L.

Additive/s in Media (µM)	Number of Nodes/culture Mean ± SE	Shoot length(m) Mean ± SE	Q Calli (mm) Mean ± SE
BPA			
0.5	2.75± 0.2	16.35± 1.3	9.10± 0.7
1.0	3.05± 0.3	14.90± 1.2	11.30± 1.0
2.0	4.55± 0.4	27.65± 3.3	18.50± 1.5
5.0	6.00± 0.3	52.90± 5.3	18.75± 0.9
Kn			
0.5	1.10± 0.1	7.90 ± 0.5	0.40± 0.3
1.0	1.15± 0.1	9.60± 0.5	0.90± 0.4
2.0	1.95± 0.2	19.20± 2.9	6.40± 0.6
5.0	3.15± 0.3	31.30± 3.4	8.25± 0.6
Zin			
0.5	4.45± 0.4	17.80± 2.0	6.90± 0.6
1.0	5.45± 0.4	18.45± 1.8	8.15± 0.3
2.0	6.85± 0.4	28.45± 2.0	9.40± 0.4
5.0	7.50± 0.3	35.30± 2.1	10.70± 0.8
2-Ip			
0.5	4.25± 0.3	38.90± 2.8	8.15± 0.4
1.0	4.35± 0.3	34.65± 3.2	7.95± 0.5
2.0	3.60± 0.3	18.95± 2.0	5.90± 0.6
5.0	3.20± 0.4	15.90± 1.6	8.55± 0.9
BAP			
0.5	5.75± 0.5	51.05± 6.7	8.70± 0.4
1.0	6.45± 0.3	42.30± 2.2	7.60± 0.3
2.0	6.65± 0.3	28.65± 2.0	6.85± 0.4
5.0	5.40± 0.4	43.15± 4.7	9.90± 0.4
Control	1.90± 0.3	9.30± 1.0	0.00± 0.0

The plants produced on MS medium supplemented with 0.5 µM to 1.0 µM 2-iP showed optimum growth with nearly similar growth pattern consisting of 4–5 nodes and 34–38 mm shoot length with 7-8 Q (mm) calli. In MS medium with 2.0 µM to 5.0 µM 2-iP, the formation of nodes as well as elongation of shoots were decreased. In MS medium supplemented with 0.5 µM BAP showed satisfactory results for multiplication of shoots with 5.75 nodes and 51.05-mm shoot length with 8.70 Q (mm) calli. In MS Medium with 1.0 µM BAP 6.45 nodes and shoot length 42.30-mm was achieved. Similarly, in the MS medium with 2.0 µM BAP, substantial node formation 6.65 and elongation of shoots 28.65 mm were observed. In another MS medium with 5.0 µM BAP the shoot length 43.15 -mm and multiplication of nodes 5.40 were observed (Table 1).

4. DISCUSSION

The number of elongating shoots were always higher on MS medium supplemented with BPA 5.0µM. In this experiment, the effect of Kn showed scarce response for the multiplication of shoots as well as node elongation. MS medium with 5.0 µM Zin showed the best node multiplication among all the above mentioned concentrations. Similarly, Wawrosch et al. [8] propagated Lilium nepalensis D.DON on MS medium supplemented with 20µM Zin using longitudinally split shoot halves. Maruyama and Ishii [9] regenerated Guazuma ulmifolia Lam. from shoots taken from five-month-old potted seedlings on WPM containing 1.0 mg/l Zin.

The shoot length elongation and node multiplication on MS medium with all the above used concentrations of 2-iP were not substantial. Whereas, Cuenca and Marco [10] propagated Salvi auaucoana from apical and nodal segments on MS medium supplemented with 4.9 µM 2iP of 6-r, r-dimethylallyl aminopurine. Similarly, Mc Cown and Lloyd [11] established Rhododendron *in vitro* culture on WPM using 8 µM 2-iP, whereas Guerin & Kirby [12] induced embryo suspension masses by culture of matured zygotic embryo isolated from Frasir fir (Abies fraseri) in a half strength MS medium in combination with

Verhagen and Wann medium containing 10 µM 2-iP.

Plants produced on MS medium with all the above mentioned concentrations of BAP were rigid and easy to propagate where the best multiplication was recorded on MS medium supplemented with 0.5 µM BAP. Gupta et al. [13] in an experiment were able to achieve multiple shoots of Lippia alba CV both from shoot tips and nodal segments on MS medium containing 2 mg/l BAP, but Krisnan and Seeni [14] micropropagated Woodfordia fruticosa (L.) Kurz, from shoot tip culture on Schenik and Hilderbrandt [15] medium with 0.2 mg/l BAP. Similarly, Berger et al. [16] induced shoots from shoot tip of Swartzia madagascarensis on agar-solidified MS medium containing 2.2 µM BA. Chen et al. [17] showed that the micropropagation and microtuburation of Dioscorea nipponica Makino on benzylaminopurine in the range of 0.5 -2.0 mg/l and naphthalene acetic acid 0.5 – 2.0 mg/l. Singh [18] propagated *Bauhinia purpurea* L. from nodal culture in MS medium supplemented with Benzylpyranyladenine and indole-3-acetic acid. Proliferated shoots were rooted easily on half strength MS medium supplemented with 1% sucrose. Similarly, in a study, Suwal et al. [19] were able to initiate rooting in Dalbergia sissoo on half strength MS medium.

5. CONCLUSION

It is evident from the current investigation that the micropropagation protocol on MS medium with cytokinin BPA 5.0 µM can be used for multiplications of shoots and 5.0 µM Zin can be used for the node multiplications of B. purpurea plants.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

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

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Manandhar NP. Plant and people of Nepal. Timber Press, Oregon, USA; 2002.
2. Kumar T, Chandrashekar KS. *Bauhinia purpurea* L. A review of its ethnobotany, phytochemical and pharmacological profile. Research Journal of Medicinal Plants. 2011;5:420–431.
3. Pettit GR, Numata A, Jwamoto C, Usami M, Yamada T, Oshishi H, Cragy GM. Anti neoplastic agents, isolation and structure of bauhinastatins 1–4 from *Bauhinia purpurea*. Journal of Natural Products. 2006;69:323–327.
4. Kumar A. Micropropagaation of a mature leguminous tree - *Bauhinia purpurea*, Plant Tissue culture and Organ Culture. 1992;31:257-259.
5. Dhar U, Upreti, J. *In vitro* regeneration of a leguminous liana (*Bauhinia vahlii* wight and Arnott). Plant Cell Reports. 1999;18:664–669.
6. Mathur J, Mukunthakumar S. Micropropagation of *Bauhinia variegata* and *Parkinsonia aculeata* from nodal explants of mature trees. Plant Cell Tissue and Organ Culture. 1992;28:119–121.
7. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures Physiol. Plant. 1962;15:473-497.
8. Wawrosch C, Malla PR, Kopp B. Clonal propagation of *Lilium nepalensis* D. Don a threatened medicinal plant of Nepal. Plant Cell Reports. 2001;20:285-288.
9. Maruyama E, Ishii K. Tissue culture of West Indian Elm (*Guazuma ulmifolia* Lam.), A Multi-Purpose Tree in the Tropical American Region. Proc. Inter. Workshop Bio Refer, Manila. 1998; 139-144.
10. Cuenca S, Marco JBA. *In vitro* propagation of two Spanish endemic species of Salvia through bud proliferation. In Vitro Cell Dev. Biol. Plant. 2000;36:225-229.
11. Mc Cown B, Lloyd GB. A survey of the response of Rhododendron to in vitro culture. Plant Cell Tissue Culture. 1983;2:77-85.

12. Guerin TG, Kirby EG. Induction of embryogenesis in cultured mature zygotic embryos of *Abies fraseri* (Pursh) Poir. Plant Cell tissue and Organ Culture. 1997;49:219-222.
13. Gupta SK, Khanuja SPS, Kumar S. *In vitro* micropropagation of *Lippia alba*. Current Science. 2001;81(2):206-210.
14. Krishnan PN, Seeni S. Rapid micropropagation of *Woodfordia fruticosa* L. Kurz. (Lathyraceae), a rare medicinal plant. Plant Cell Reports. 1994; 14:55-58.
15. Schenk RU, Hildebrandt AC. Medium and techniques for induction of growth of monocotyledonous and dicotyledonous plant cell culture. Can. J. Bot. 1972;50:166-204.
16. Berger K, Schaffner W. *In vitro* propagation of the leguminous tree *Swartzia madagascariensis*. Plant Cell Tissue and Organ Culture. 1995;40:289-291.
17. Chen FQ, et al. The effect of plant growth regulators and sucrose on micropropagation and microtuberization of *Dioscorea nipponica* Makino. Journal of Plant growth Regulators. 2007;26: 38-45.
18. Singh BMS. Effects of N-Benzyl-9-2-tetrahydropyranyl and indole-3-acetic acid on *in vitro* culture of *Bauhinia purpurea* L. International Journal of Scientific Research and Technology. 2019;6(3):238-242.
19. Suwal B, Karki A, Rajbhandary SB. The *in vitro* proliferation of forest trees 1. Dalbergia sissoo Roxb. Ex DC. Sivae Genetica. 1988;37(1):26-28.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/58906