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Research Article

ESTABLISHMENT OF LONG TERM PROLIFERATING SHOOT CULTURES OF *COMMELINA BENGHALENSIS* L. A MEDICINAL PLANT

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ABSTRACT

Regeneration of Plantlets through nodal explants has been achieved in medicinal plant *Commelina benghalensis* of commelinaceae, belongs to monocot. Green house grown explants were used to control browning due to phenolic exudation. Plantlets obtained by nodal explants on Murashige and Skoog's (MS) medium fortified with 1.0 mg l^{-1} BAP (6, Benzyl amino purine) produced 06-10 multiple shoots. Cut ends of nodal stem segments rapidly turned brown and cultures failed to establish. When 50 mg l^{-1} ascorbic acid and 25 mg l^{-1} Polyvinyl pyrrolidone (PVP) were added to the medium, explants remained healthy, and cultures were established. Rooting of micro shoots was achieved on half or full strength MS medium with 0.5-2.0 mg l^{-1} NAA (naphthalene acetic acid) for 15 days. Regenerated plantlets acclimatized showed normal morphological characteristics when transferred to soil. Survival of transplants was 95-99 % under green house condition.

Keywords: *Commelena benghalensis*, medicinal plant, Plantlets and Regeneration.

INTRODUCTION

Commelina benghalensis L. is an annual or perennial herb of commelinaceae, commonly called Bengal day flower, believed to be native only to tropical Asia and Africa. In Africa and India the leaves and stems are chopped and cooked as vegetables and also used as feed for livestock. Different components are also used for mouth thrush (Ssenyonga and Brehony, 1993), wound healing (Burkill 2000), insanity(Tabuti *et al.,*2003), inflamation of the conjunctiva, psychosis, epilepsy, nose blockage in children (Okello and Ssegawa, 2007), demulcent, refrigerant, laxative, bitter, emollient, depressant and for the treatment of leprosy in India (Jayvir *et al*., 2002), diuretic, febrifuge, anti-inflammatory and laxative (Deyuan and Robert, 2000; Hong and Defillipps, 2000), laxative and to cure inflammations of the skin as well as leprosy (Qaiser and Jafri, 1975). *In vitro* technique proved as a potential tool for conservation and propagation of medicinal plants. Establishment of Micro propagation protocol have been reported in the medicinal plants viz. *Physalis peruviana* L (Ramar, *et al.,* 2014), *Cochlospermum religiosum* L. (Pareek *et al.,* 2014), *Centella asiatica* L. (Suryakala and Archana. 2014) *Withania somnifera* (Pramila, *et al*. 2014) and *Eclipta alba* L.(Ragavendran, *et al*. 2014).

Until now, there are no reports available on micro propagation and efficient *in-vitro* regeneration of this valuable medicinal plant. Hence, the present work has been undertaken in *Commelina benghalensis.*

MATERIALS AND METHODS

Plants were collected in and around Mysore University campus and were grown in the departmental garden. Newly formed healthy nodes were collected to prevent microbial contamination and cut to approximately 1 cm. Explants were washed thoroughly under running tap water to remove the traces of dust and surface sterilized with 0.1%(w/v) $Hgcl_2$ for 2 minutes and rinsed with sterile distilled water for five times. The Nodal explants were inoculated on Murashige and Skoog's medium supplemented with various concentrations of 2,4-D,BAP,NAA,IAA,IBA and KN individually or in combinations. The cultures were maintained

at 25± 2^0 c under 16/8 hour light and dark conditions.

For experimental trials, culture media was prepared using Murashige and Skoog's medium containing 3% sucrose and 0.8% (w/v) agar. The pH of the media was adjusted to 5.8 with 0.1 N NaOH after supplemented with 2,4-Dichlorophenoxy acetic acid (2,4-D) at 0.5 and 2.0 mgl^{-1} or with Napthalene-3-acetic acid (NAA) at 0.5 and 2.0 mg1^{-1} either singly or in combination with 6-benzylaminopurine (BAP) at1.0-3.0 mgl^{-1}.The prepared media was poured into a culture tubes or flasks and plugged with nonabsorbent cotton wrapped in two layers of cheese cloth then autoclaved at 121°C for 15 minutes.

Each culture tube or flask containing medium was inoculated with one nodal explant vertically with the proximal region facing up. All cultures were maintained at 25 ± 2°C under white fluorescent light ($65\ \mu E\ m^{-2}\ sec^{-1}$) with a 16-hour photoperiod. Elongated multiple shoots obtained after 28 to 35 days on the regeneration medium were separated and used as a best source of nodal explants to establish long term cultures and were transferred to half strength MS basal medium fortified with NAA and IBA 0.5 to 2 mgl^{-1} for rooting.

Shoots of 6.0 to 7.0 cm having seven to eight leaves obtained after 21 days of culture on the rooting medium were removed from the media and washed one or two times in running tap water to remove agar before being transferred to plastic pots filled with 1:1 sand and soil (v:v) mixture. The potted plantlets were hardened in the greenhouse condition.

STATISTICAL ANALYSIS

All data were subjected to analyse using SPSS Package 11.5 . 12 explants were used in each of two replicates for each treatment and the experiment was repeated twice.

RESULTS AND DISCUSSION

MS media is the best culture medium for the establishment of culture in *Commelina benghalensis* compared to B5 Media. Nodal explants inoculated on basal MS medium failed to produce any response. MS medium containing 2,4-D,IAA, NAA, BAP and KN etc in the range 1.0-5.0 mgl^{-1} were tested for shoot formation. The combination of BAP and NAA, BAP and 2,4-D promotes both shoot and root formation but size and number of roots are not satisfactory. Cut ends of nodal stem segments rapidly turned brown and cultures failed to establish (Fig A). Explants remained healthy and cultures were established when 50 mg l^{-1} ascorbic acid and 25 mg l^{-1} Polyvinyl pyrrolidone (PVP) were added to the medium. Shoots after their initial proliferation were sub-cultured on same fresh medium after every 5-7 days also helps in prevention of browning of media by phenolic exudation. Among the concentration and combination of hormones used best response was observed on medium containing BAP alone compared to KN. BAP showed average number of shoots 10±0.74, shoot length 9±0.22 cm on its 1.0 mgl^{-1} concentration (Table 1, Fig. B), which is parallel with the earlier reports on *Zingiber officinale* (Balachandram, and Bhat, 1990), *Houttuynia cordata* (Handique and Bora 1999), *Sida cordifolia* (Sivanesan and Jeong, 2007), *Morus alba* (Balakrishan *et al.,* 2009) and *Azadirachta indica* (Arora *et al.,* 2010). Nodal explants have been reported as the best source for the multiple shoot induction in case of medicinal plants such as *Boerhaavia diffusa* L. (Roy 2008*) Rhinacanthus nasutus* (Johnson, *et al.,* 2006) and *vitex negundo* (Vadawale *et al.,* 2006)

Since the plant is seasonal, elongated multiple shoots were separated and used as source of nodal explants to establish long term cultures throughout the season in pure form.

Different concentration of Auxins induced rooting when micro shoots were transferred to the medium containing half or full strength of MS solid or liquid medium. The best rooting response, however, was observed on medium containing 0.5mgl^{-1} NAA, where roots measuring 6.9 ± 0.75 cm were formed (Table 2, Fig C). *In vitro* rooted plantlets were initially acclimatized in culture room conditions before being transferred to greenhouse condition. There was an increase in length of shoots and new leaves emerged which expanded quickly with the survival rate of 95 - 99 percent (Fig D).

CONCLUSION

Since the plant is a weed, most of the farmers destroying the plants without knowing the medicinal value and may contaminate with pesticides or micro organisms. In the present work maximum number of multiple shoots (6-10 shoots per explants) were obtained from Nodal explants of *Commelina benghalensis*. There is no much work has been done on plant tissue culture of this medicinally important plant for mass propagation. Thus, this protocol can be used to exploit on a large scale for commercial purpose in the pure form.

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Table 1: The Effects of growth regulators on shoot induction in *C.benghalensis* nodal explants

Growth regulators ($mg1^{-1}$)	% Shoot formation	Shoot per explants	shoot length of of excised shoot (cm)
BAP			
0.0	00.0	00.00	00.00
0.5	39.0	02±0.41	1.4±0.05
1.0	90.0	10±0.74	9.0±0.22
1.5	65.0	06±0.21	8.2±0.45
2.0	56.0	04±0.84	5.2±0.13
2.5	48.0	04±0.26	3.5±0.16
3.0	27.0	03±0.38	1.9±0.82
KN			
0.0	00.0	00±0.0	00±00
0.5	36.0	00±0.0	00±00
1.0	42.0	01±1.2	1.7±0.82
1.5	53.0	02±0.5	1.9±0.97
2.0	60.0	04±0.5	2.0±0.63
2.5	51.0	02±0.8	2.5±0.23
3.0	23.0	01±0.5	1.6±0.05
BAP +2,4-D			
0.5+0.5	35.33	00±0.0	00±00
1.0+0.5	39.0	01±0.6	00±00
1.5+0.5	68.0	03±2.4	02.3±0.41
2.0+0.5	76.0	06±0.5	03.0±0.06
2.5+0.5	76.0	03±0.5	03.1±0.82
BAP+NAA			
0.5+0.5	28.31	02±0.6	01.3±0.06
1.0+0.5	34.38	03±0.9	01.3±0.16
1.5+0.5	48.14	03±1.6	01.6±0.45
2.0+0.5	64.13	04±1.6	04.8±0.17
2.5+0.5	72.00	05±0.5	03.8±0.16

Values are mean ± SE of the two experiments with 12 explants each

Table 2: Effect of different auxins on *in vitro* rooting of micro shoots of *C.bengalensis* Auxin concentration (mg^{-1})

IAA	IBA	NAA	% of response	Root length (cm)
0.00	0.0	0.0	00	00
0.25	0.0	0.0	10	0.1±0.2
0.3	0.0	0.0	10	0.1±0.2
0.5	0.0	0.0	12	0.2 ±0.5
1.0	0.0	0.0	10	00
0.0	0.00	0.0	00	00
0.0	0.25	0.0	00	00
0.0	0.3	0.0	10	0.7±0.24
0.0	0.5	0.0	30	0.7±0.24
0.0	1.0	0.0	45	0.9 ± 0.85
0.0	0.0	0.00	00	00
0.0	0.0	0.25	54	3.0±0.42
0.0	0.0	0.3	68	4.0±0.16
0.0	0.0	0.5	100	6.9 ± 0.75
0.0	0.0	1.0	72	3.9 ± 0.18

Values are mean±SE of the two experiments with 12 explants each

Commelina bnghalensis **A.Media showing browning due to phenolics B. Multiple Shoot proliferation from nodal explant on MS medium supplemented with 1 mg1^{-1} BAP after 4 weeks of culture. C. Formation of roots from regenerated shoots cultured on ½ liquid MS medium supplemented with 1.0 mgl^{-1} NAA. D.Plantlets developed in vitro, transferred to pot.**

REFERENCES

1. Ssenyonga M and Brehony E. Herbal medicine-its use in treating some symptoms of AIDS. Int Conf. AIDS, 1993;9:75-75.
2. Burkill HM. The useful plants of west tropical Africa. Royal Botanic Gardens, Kew. 2000;1:429-430.
3. Tabuti JRS Dhillion SS and Lye KA. Traditional medicine in Bulamogi county, Uganda: its practitioners, users and viability. J Ethnopharmacol. 2003;85:119–129.
4. Okello J and Ssegawa P. Medicinal plants used by communities of ngai subcounty,apac district, northern uganda. Afr J Ecol. 2007;45:76-83.
5. Jayvir A Minoo P Gauri B and Ripal K. Nature Heals: A glossary of selected indigenous medicinal plant of India.2nd Ed., SRIST Innovations, Ahmedabad, India. 2002;22.5.
6. Deyuan H and Robert AD. Commelina Diffusa. In: Flora of China, Wu ZY Raven PH and Hong DY. (Eds.). Botanical Garden Press, Beijing. 2000;24:36.

7. Hong DY and DeFilipps RA. Commelinaceae. In: Wu ZY and Raven PH (eds). Flora of China (Flagellariaceae through Marantaceae) Science Press, Beijing. 2000;24:19-39.
8. Qaiser M and Jafri SMH. Commelina benghalensis. In: Flora of Pakistan, (Ali SI,Qaiser M,eds). University of Karachi & Missouri Botanical Garden, St. Louis, 1975;84:10.
9. Ramar K, Ayyadurai V and Arul prakash T. In Vitro Shoot Multiplication and Plant regeneration of Physalis peruviana L. An Important Medicinal Plant. Int J Curr Microbiol App Sci. 2014;3(3):456-464.
10. Apexa Pareek, Alok Sharma and Payal Lodha. Multiple Shoots Regeneration Through Nodal Segment in Cochlospermum religiosum (L.) Alston: A Medicinal Endangered Plant. Int J Pharm Sci Rev Res. 2014;29(2):107-110.
11. Suryakala Gandi and Archana Giri. Standardizatiion protocol for high frequency seed germination and direct regenrationin centella asiatica L. An important medicinal plant. Journal of Chemical and Pharmaceutical Research. 2014;6(10):224-230.
12. Pramila Bhuria, Yogendra Singh, Sharad Tiwari and Meenakshi Chaturvedi. Studies On multiple Shoot Regeneration From Nodal Explant Of Withania somnifera(L.) Indian Res. J Genet and Biotech. 2014;6(3):521-525.
13. Ragavendran C Kamalanathan D and Natarajan D. A Rapid Micropropagation of nodal explants of Eclipta alba (L.). A Multipurpose Medicinal Herb Research in Biotechnology. 2014;5(2):06-12.
14. Balachandran SM and Bhat SR. In vitro clonal multiplication of turmeric(curcuma Spp.) and gingiber (Zingeiber officinale Rose). Plant cell Rep. 1990;8:521-524.
15. Handique PJ and Bora P. In vitro regeneration of medicinal plant Houtuynia cordate Thunb. from Nodal explants. Cur Sci. 1999;76:1345-1247.
16. Sivanesan I and Jeong BR. Direct shoot regeneration from nodal explants of Sida cordifolia Linn. In vitro Cell. Dev Biol Plant. 2007;43:436-441.
17. Balakrishnan V, Latha RM, Ravinder KC and Robinson PJ. Clonal propagation of Morus alba L. through nodal and axillary bud explants. Botany Research International. 2009;2:42-49.
18. Arora K, Sharma M, Srivastava J, Ranade SA and Sharma AK. Rapid in vitro cloning of a 40-year-old tree of Azadirachta indica A. Juss. (Neem) employing nodal stem segments. Agroforest Syst. 2010;78:53–63.
19. Roy PK. Rapid Multiplication of Boerhaavia diffusa L. Through In vitro Culture of Shoot tip and Nodal Explants. Plant Tissue Cult and Biotech. 2008;18(1):49-56.
20. Johnson M, Vallinayagam S, Manickam VS and Seeni S. Micropropagation of Rhinacanthus nasutus (L.) Kurz- A medicinally important plant. Phytomorphology. 2006;52:331-336.
21. Vadawale AV Barve DM and Dave AM. In vitro flowering and rapid propagation of Vitex negundo L.- A medicinal plant. Indian J Biotechnol. 2006;5:112-116.