

PERSPECTIVES IN LIFE SCIENCES



Editor: Dr. M.V. Sudhakaran



PERSPECTIVES IN LIFE SCIENCES

Editor
Dr. M. V. Sudhakaran



University Grants Commission



UGC-Human Resource Development Centre
University of Calicut



Sahithya Pravarthaka Co-operative Society,
Kottayam, Kerala

(English)

PERSPECTIVES IN LIFE SCIENCES

SCIENCE

© UGC-HRDC University of Calicut

Chief Editor

Dr. M. A. Joseph

Editor

Dr. M. V. Sudhakaran

Published by

UGC-HRDC University of Calicut

Malappuram, Kerala

In association with

Sahithya Pravarthaka Co-operative Society Ltd. No. 2458

Kottayam, Kerala

Email: spcsktm@gmail.com

Price: Rs. 650.00

Cover Design

Vinod Kumar C. S.

First Published December 2019

Printed at

Romanson Print House, Thiruvananthapuram

ISBN 978-93-88992-90-9

978000031767

Sales Department

National Book Stall

Thiruvananthapuram-Kollam-Alappuzha-Thiruvalla
Kottayam -Thodupuzha-Ernakulam (Marine Drive)-Irinjalakkuda
Thrissur-Palakkadu-Kozhikkode-Kalpatta-Kannur

www.nationalbookstall.com

S 10220

B 6267

128/19-20

1-80

Sl.	Contents	Page No.
1.	Genetic consequences and cost of inbreeding in some population groups of Kerala Sudhakaran M.V.	1-10
2.	A study on the grasses of Kalarikunnu hills, Kozhikode District of Kerala Abhilash. E.S. & Aparna Rajan	11-16
3.	Mechanism of anthelmintic action of triclabendazole in tropical liver fluke, <i>Fasciola gigantica</i> Ahammed Shareef P.A. & Abidi S.M.A.	17-33
4.	Toxicological effects of amoxicillin on <i>Oreochromis niloticus</i> Ambili T.R. & Anjitha K.M.	34-42
5.	Nitrogenase and phosphatase activity along with nutrient status of mycorrhizal <i>vigna unguiculata</i> , co-inoculated with rhizobium and a phosphate solubilizing bacteria Anilkumar K.K.	43-57
6.	Ectoparasitic tick infestation of domesticated cows of a semi urban village in Malappuram District, Kerala Aswathi P. & Princy P.	58-62
7.	Micropropagation of <i>Plectranthus zeylanicus</i> Benth. Betty K.P.	63-69
8.	A study on drought responses of selected grasses Biby Annet Baiju	70-76
9.	Ethnobotanical documentation of traditional knowledge about medicinal plants used by <i>Paniyans</i> of Vaniyampuzha, Nilambur Taluk, Malappuram, Kerala Binu Thomas & Manju S.P.	77-109

MICROPROPAGATION OF *PLECTRANTHUS* *ZEYLANICUS* BENTH.

Betty K.P.

**Department of Botany, Sacred Heart College,
Chalakydy – 680307, Thrissur, Kerala, India**

Introduction

Living plant cells are abundantly equipped with the raw materials for building thousands of compounds that have medicinal properties such as glycosides, alkaloids, essential oils, tannins, flavanoids, antibiotics *etc.* Since natural flora represent an unlimited source of several novel compounds, the conservation and management of natural resources need our prime consideration.

The collection of medicinal and aromatic plants scattered in the natural flora is troublesome and also cannot cope up with the ever increasing and changing condition made by the indigenous systems of medicine and also by the modern pharmaceutical industries. Regular and sustained supply of these plants in accord with the mounting demand necessitates the domestication and propagation of them in a large scale. In this imperative context, the recent exciting developments in biotechnology have come as a blessing. Fast changing research scenario globally has established plant tissue culture as a fascinating tool of biotechnology. Micropropagation seems to be an apt answer for increasing efficiency and scale up of plant production.

The genus *Plectranthus* in the family Lamiaceae is a large and widespread genus with about 80 species having several ethno-botanical uses. *Plectranthus zeylanicus* Benth. [synonym. *Coleus zeylanica* (Benth.) Cramer] is a native of Sri Lanka commonly cultivated as a medicinal plant (Nayar *et al.*, 2006). The vernacular name of the plant is Iruveli in

Malayalam. The plant is profusely branched, semi-succulent, strongly aromatic, softly tomentose herb with fibrous roots, leaves slightly fleshy, ovate or orbicular, and flowers small, blue or purple in terminal, paniced thyrsus (CSIR, 2004). The herb is identified as the source of an Ayurvedic drug Hribera (Iruveli, Valakah, Balam) in Kerala. According to Bhavaprakasanighantu, this drug is cooling, light, carminative and toxic and cures dyspepsia, indigestion, dysentery, vomiting, thirst, fever, dermatitis, ulcers and bleeding disorders. The entire plant is used in medicine. Gandhatailam and Eladitailam are some of the preparations using the drug (Sivarajan and Balachandran, 2002). The juice of the stem and leaves mixed with honey is taken as a remedy for diarrhoea (CSIR, 2004). It is a chief ingredient of many ayurvedic preparations like iruvelikashayam, devashtagandha, snanachooram etc.

In the present study an attempt is made for the micropropagation of *Plectranthus zeylanicus* through direct multiplication as well as callus mediated regeneration using the formulation described by Murashige and Skoog (MS medium, 1962).

Results and Discussion

Murashige and Skoog medium augmented with varied hormonal combinations resulted in the establishment of *in vitro* multiplication of *Plectranthus zeylanicus*. Among the entire explants tested, nodal cuttings and shoot apices showed better response. The varying concentrations of auxins and cytokinins influenced the growth and regeneration of explants in different levels. The cultured explants regenerate shoots directly or through callus phase. Emergence of shoots directly from cultured explants will be useful in the propagation of true-to-type plants and that from the callus will induce variations.

The level and kind of plant growth regulators included in the culture medium largely determine the success of tissue culture. Direct regeneration of multiple shoots from nodal and shoot tip explants occurred on MS medium supplemented with different concentrations of NAA (1-3 mg/l) along with BAP (0.5-3.0 mg/l). Maximum number of shoots was obtained

from nodal segments on MS medium fortified with BAP (3 mg/l) and NAA (1 mg/l). 90% of the cultures produced 5-10 shoots from each explant. About 10% of cultures produced 1-2 shoots per explant. The hormonal combination of IAA (1-2 mg/l) and BAP (1 mg/l) as well as NAA (1 mg/l) and KIN (2 mg/l) showed multiple shoot induction. But the frequency of shoots and percentage of initiation was lower than the combination of NAA and BAP. When KIN and BAP were applied singly, it resulted in the development of single shoots from nodal explants. For shoot apex, multiple shooting was obtained with BAP and NAA at an average proportion. Skoog and Miller (1957) reported that shoot bud regeneration depends on quantitative interaction between various growth regulators, *viz.*, auxins and cytokinins. The superiority of BAP over other cytokinins for multiple shoot formation has been reported in many plants (Lundergan and Janic, 1980; Rahman and Blake, 1988; Sen and Sharma, 1991).

When nodal cuttings and shoot apices were cultured on MS medium supplemented with NAA and BAP, callus growth was initiated in about 10 days and rapid growth followed for majority of cultures. The maximum response was observed on MS medium with NAA 1.5 mg/l and BAP 0.5 mg/l, where callus production was 90%. Upon subculturing in the same medium profuse callusing was resulted. Nodal segments exhibited the highest (up to 90%) callusing response after 4 weeks in incubation media. The callus growth from shoot apex was also significant (up to 80%). Of the 2 auxins tested, NAA proved better than 2,4-D in inducing callus and upon supplementing BAP, the percentage and amount of callus formation was increased. The explants produced white friable callus in medium with NAA 1 mg/l and BAP 0.5 mg/l and greenish soft callus with 2,4-D 2 mg/l after a period of 4-5 weeks in culture. According to Hakman and Fowke (1987) and Von and Woodward (1988) presence of auxin together with cytokinin promotes the induction and formation of organogenic callus. NAA when used singly (1 mg/l) formed roots along with small amount of callus. Medium with IAA and BAP produced very little callus at the cut end of the nodal explants and resulted in the axillary bud elongation.

Leaf explants inoculated on media with 2,4-D (1.5 mg/l) and BAP (0.5 mg/l) produced callus but the intensity was less. With NAA (1-1.5 mg/l) and BAP (0.5 mg/l), white friable callus was obtained. On subculturing they failed to regenerate with different hormonal combinations. Direct regeneration of plantlet with roots from leaf explants was noticed in the hormonal proportion of 2 mg/l BAP and 1.5 mg/l NAA. The other hormonal combinations produced only swelling and crumpling of the tissue in the case of leaf explants. This is in agreement with the hypothesis that the balance of growth regulators as well as their concentration is critical in determining the direction of morphogenesis (Sharief and Jagadishchandra, 1999). Negative response was noticed in all hormonal combinations when internodes were used as explant.

For plant regeneration organogenic calli were transferred to the development media. Shoot proliferation and multiplication was observed from the totipotent calli, in the suitable hormone supplemented medium within 3 weeks of culture. The optimum growth regulator combination for shoot regeneration among all the treatments was MS medium with 1.5 mg/l NAA and 1-2 mg/l BAP. Heszky *et al.* (1991) identified the influence of an optimum combination of auxin and cytokinin in the expression of morphogenic potential. The age of the callus influenced the regeneration capability. Younger calli (4 weeks old calli) showed better morphogenic performance than the old calli (6-7 weeks old calli).

The shoots measuring about 5 cm in height were separated and inoculated for rooting on different concentration of auxins - NAA, IAA and IBA. IBA was clearly more effective in promoting root induction than NAA or IAA. The optimum medium for rooting was found to be having 1 mg/l IBA on which 95% of the regenerated shoots developed roots with an average number of 10-15 roots per shoots within 10 days. The relative levels of auxins have been known to greatly influence morphogenic responses like rooting (Sitborn *et al.*, 1993). The observation of the performance of IBA in root induction than other auxins like IAA and 2,4-D (Amin and Rahman, 1994) emphasized the fact that auxin types differ in their morphogenic ability and organogenic effect on plant tissues in culture. The promotory role of IAA in rooting was noticed in a number of

species (Thorpe, 1978). IAA and IBA were known to have root induction capacity in the experiments by Purohit *et al.* (1995).

Plantlets thus obtained were transferred to cups with 1:2 mixtures of soil and sand and acclimatized for a week under humid conditions and was found to be with 90% of survival efficiency. They were later successfully adapted to field conditions. The regenerated plants were free of any noticeable phenotypic variability.

Clonal multiplication of *Plectranthus zeylanicus* through artificial culture medium provides an opportunity to raise a number of plants within a short time, in a limited space to meet the ever-increasing demand of the natural products.

References

- Amin, M. N. and Rahman, M. A. (1994). *In vitro* regeneration from seedling explants of a subtropical tree – monkey jack. VIIIth Intl. Cong. Plant Tissue and Cell Cult., Firenze, Italy.
- Ammirato, P. V. (1983). Embryogenesis. In: Evans, D. A., Sharp, W. R., Ammirato, P. V. and Yamada, J. (Eds.) *Handbook of Plant Cell Culture*. Macmillan, New York. vol. I, pp. 82 - 123.
- Bajaj, Y. P. S., Furmanowa, M. and Olszowska, O. (1988). Biotechnology of the micropropagation of medicinal and aromatic plants. In: Bajaj, Y. P. S. (Ed.) *Biotechnology in Agriculture and Forestry*. Springer Verlag, New York. vol. 4, pp. 60 - 103.
- CSIR. (2004). *The Wealth of India*. New Delhi. vol. 2, p. 156.
- Hakman, I. and Fowke, L. C. (1987). Somatic embryogenesis in *Picea glauca* (white
- Heszky, L. E., Nam, L. S., Simon, I. K., Kiss, E., Lokos, K. and Quang, D. (1991). *In vitro* studies on rice in Hungary. In: *Biotechnology in Agriculture and Forestry*. Springer Verlag, Berlin. pp. 619 - 641.
- Lundergan, C. A. and Janic, J. (1980). Regulation of apple shoot proliferation and growth *in vitro*. *Hortic. Res.*, 20: 19 - 24.

- Murashige, T. (1980). Plant growth substances in commercial uses of tissue culture. In: Skoog, F. (Ed.) *Plant Tissue Culture*. Springer Verlag, Berlin. pp. 426 - 434.
- Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*, 15: 473 - 497.
- Nayar, T. S., Rasiya Beegam, A., Mohanan, N. and Rajkumar, G. (2006). *Flowering Plants of Kerala - A Handbook*. TBGRI, Thiruvananthapuram.
- Purohit, M., Pande, D., Datta, S. and Srivastava, P. S. (1995). Enhanced xanthotoxin content in regenerating cultures of *Ammi majus* and micropropagation. *Planta Med.*, 61: 481 - 482.
- Rahman, M. A. and Blake, J. (1988). Factors affecting *in vitro* proliferation and rooting of shoots of jack fruit (*Artocarpus heterophylla* Lam.). *Plant Cell Tissue Organ Cult.*, 13: 179 - 187.
- Rout, G. R. and Das, P. (1997). Techniques of micropropagation *in vitro*. In: Bose, T. K., Mitra, S. K., Sadhu, M. K. and Das, P. (Eds.) *Propagation of Tropical and Sub-tropical Horticultural Crops*. Kalyani Publishers, Calcutta. pp. 105 - 116.
- Rout, G. R. and Das, P. (2004). Role of growth regulators on micropropagation of medicinal plants: a review. In: Govil, J. N. Kumar, P. A. and Singh. V. K. (Eds.) *Recent Progress in Medicinal Plants. Biotechnology and Genetic Engineering*. Stadium Press, U.S.A. vol. 4, pp. 327 - 358.
- Rout, G. R., Saxena, C., Samantaray, S. and Das, P. (1999). Rapid clonal propagation of *Plumbago zeylanica* Linn. *Plant Growth Regul.*, 28:1-4.
- Saxena, C., Rout, G. R. and Das, P. (1998). Micropropagation of *Psoralea corylifolia* Linn. *J. Medicinal Aromatic Plant Sci.*, 20: 15 - 18.
- Sen, J. and Sharma A. K. (1991). *In vitro* propagation of *Coleus forskohlii* Briq. for forskolin synthesis. *Plant Cell Rep.*, 9: 696 - 698.
- Sharief, U. M. D. and Jagadishchandra, K. S. (1999). Biotechnological approaches of aromatic trees - relevance to *Chloroxylon sweitenia* DC: Micropropagation and its phytochemical studies. In: Khan, I. A. and

- Khanum, A. (Eds.) Role of Biotechnology in Medicinal and Aromatic plants II. Ukaaz publications, Hyderabad. pp. 376 - 391.
- Sitborn, F., Ostin, A., Sundberg, B., Olsson, O. and Sandberg, G. (1993). Conjugation of indole-3-acetic acid in wild type and IAA overproducing transgenic plants and identification of the main conjugates by first-fast atom bombardment liquid chromatography mass spectrometry. *Plant Physiol.*, 101: 313 - 320.
- Sivarajan, V. V. and Balachandran, I. (2002). Ayurvedic Drugs and Their Plant Sources. Oxford and IBH publishing Co. Ltd. New Delhi. p. 498.
- Skoog, F. and Miller, C. O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. In: Biological Action of Growth Substances. XIth Symp. Soc. Expl. Biol., Cambridge Univ. Press.
- Thorpe, T. A. (1978). Physiological and biochemical aspects. In: Thorpe, T. A. (Ed.) Frontiers of Plant Tissue Culture. University of Calgary Printing Service, Calgary, Canada. pp. 49 - 58.
- Von, A. S. and Woodward, S. (1988). Organogenesis and embryogenesis in mature zygotic embryo of *Picea sitchensis*. *Tree Physiol.*, 4: 291 - 300.