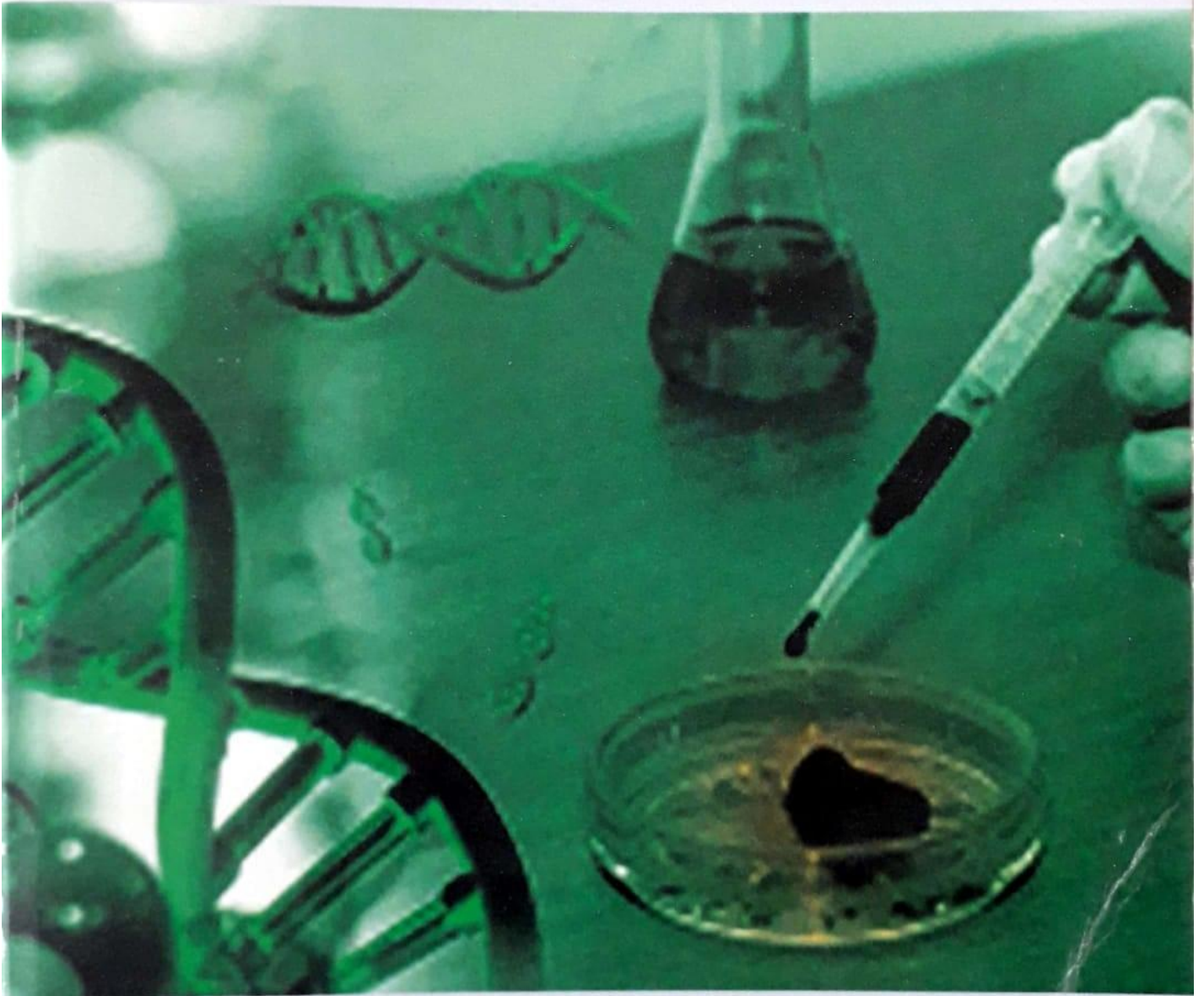


PERSPECTIVES IN LIFE SCIENCES



Editor: Dr. M.V. Sudhakaran



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MICROPROPAGATION OF *PLECTRANTHUS* *ZEYLANICUS* BENTH.

Betty K.P.

Department of Botany, Sacred Heart College,
Chalaky - 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.

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