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Micropropagation of *Operculina turpethum* (Linn.) Silva Manso. Using Cotyledonary Node Explants

[1]J. Sebastinraj, [2]S. John Britto and [2]S. R. Senthil Kumar

[1]Department of Biotechnology, Jamal Mohamed College (Autonomous), Tiruchirappalli, Tamilnadu, South India - 620020
[2]The Rapinat Herbarium and Center for molecular systematic, St. Joseph's College (Autonomous),Tiruchirappalli, Tamilnadu, South India - 620002

Abstract: *Operculina turpethum* is an endangered medicinal plant of peninsular India, threatened by overexploiation. Cotyledonary nodes (1 cm long) excised from 15-20 days old seedlings germinated *in vitro* served as explants source. The seeds were germinated on Half Strength Murashige and Skoog (HMS) medium devoid of plant growth regulators. Cotyledonary nodes were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of cytokinins (BAP and KIN) and auxin (IAA). Maximum shoot proliferation was obtained on MS medium supplemented with KIN+IAA (1.5+0.2mg/l). The highest frequency of root proliferation was on MS medium supplemented with 0.5mg/l IBA.

Key words: Cotyledonary node · Micropropagation · Multiple shoots · Endangered medicinal plant

INTRODUCTION

The genus *Operculina* (Fam. *Convolvulaceae*) consists of about 25 species occurring in tropical areas worldwide. *Operculina turpethum* (L.) S. Manso, a perennial herbaceous vine, is distributed in India, China and Australia [1]. The roots and stems of *O. turpethum* have traditionally been used in Indian medicine to treat a wide range of ailments, such as tumors, burning diseases, jaundice, paralysis and pain in the joints and muscles [2]. Furthermore, an *O. turpethum* extract has been used to relieve fevers and to treat anemia, splenomegaly, raised lipid levels, obesity, gastric ulcer and related gastrointestinal disturbances [3,4]. *Operculina turpethum* is a large perennial twiner of the family Convolvulaceae. It is a rare and endangered medicinal plant of peninsular India threatened by overexploitation. The plant drug known as Turpeth or Indian Jalap it was used for purgative. In constipation, it is an effective laxative. It is used in periodic fever and treatment of obesity and it is used to decrease fat. Alcoholic extract of fresh root show antibacterial activity against *Micrococcus pyogenes* and *Escherichia coli*. Young leaves and tender stems are reported to be used as vegetable in Philippines and stems are used for tying purpose [5].

Plant biotechnology holds great promise for micropropagation, conservation and enhancement of the natural levels of valuable plant secondary metabolic products and to fulfill pharmaceutical demands and reduce the *in situ* harvesting of natural forest resources [6,7]. For mass propagation of medicinal plant species in which conventional methods possess restrictions, *in vitro* multiplication provides the way out. There are many reports available about procedures on *in vitro* micropropagation of many threatened medicinal species [8,9]. *In vitro* plant regeneration is a complex phenomenon involving different biochemical mechanisms for its progression [10]. *In vitro* propagation refers to true-to-type propagation of selected genotypes using in-vitro studies. Different explants such as single cells, protoplasts, pieces of leaves or roots can be used to generate a new plant on culture media with required nutrients.

Plant tissue culture is an effective means for rapid multiplication of endangered species in which conventional methods present limitations. Established aseptic cultures and development of an efficient protocol for regenerated and multiplication of plants are required for developing invitro strategies for conservation.

Corresponding Author: J. Sebastinraj, Department of Biotechnology, Jamal Mohamed College (Autonomous), Tiruchirappalli, Tamilnadu, South India - 620020.

Attempts have been made to micropropagate endangered medicinal value species of *Crotalaria lutescens*, *Enicostemma hyssopifolium*, *Helianthemum bystropogophyllum*, *Curcuma zedoaria* [11, 12, 13, 14]. The present study was therefore undertaken with an aim of establishing an efficient protocol for *in vitro* plant regeneration from cotyledonary nodes of *in vitro* grown seedlings.

MATERIALS AND METHODS

Plant Material: Seeds of *Operculina turpethum* were collected from riverbank of Cauvery, Tamil Nadu, South India.

Reagents: All the chemicals used in this study were purchased from Hi-Media Pvt. Ltd (Mumbai, India. The chemicals used were of analytical grade.

***In vitro* Micropropagation:** The mature seeds were washed under running tap water for 5-10 min and thoroughly washed with few drops of Teepol for 5 min and then with distilled water. Then the seeds were immersed in hot water at 80°C for 2 min. Subsequently, they were sterilized inside the laminar air flow chamber with 0.1%mercuric chloride for 5 min and rinsed thoroughly with sterilized double distilled water and germinated on Half Strength MS medium [15] devoid of plant growth regulators. Cotyledonary node segements (1cm) from 15-20 days-old seedlings were taken for *in vitro* culturing. The medium containing 3% (w/v) sucrose and concentrations of cytokinins 6-Benzylamino purine (BAP), Kinetin (KIN) (0.5-2.5 mg/l) and auxins particularly Indole-3-acetic acid (IAA) (0.1-0.5 mg/l) for raised multiple shoots. The media were adjusted to pH 5.7 followed by the addition of 0.8% (w/v) agar. Borosil glass tubes (25x150mm) each containing 15ml of the medium and capped with non-absorbent cotton and it was autoclaved at 121°C for 20 min duration under 15 Lbs psi pressure. In each tube single cotyledonary nodal explants was inoculated. The cultures were then maintained under 16hrs light provided with cool white fluorescent lamps a temperature of 25±2°C.

Induction of Rooting and Acclimatization: *In vitro* propagated plantlets (7-10 cm) having at least seven leaves and four to five nodes were excised from the shoot clump and transferred to MS medium containing various concentration of Indole-3-butyric acid (IBA) (0.1-0.9 mg/l) for rooting. After two weeks of root induction, the plantlets were removed from the culture tubes and washed thoroughly with sterile double distilled water to remove traces of agar medium and then treated with 0.1%(w/v) Bavistin (fungicide) and planted in small polycups filled with a mixture of sterile garden soil, sand and farmyard manure (2:1:1). The plants were covered by plastic bags and maintained under humidity in the culture room. Plants were then acclimatized to a reduced relative humidity by gradually opening the plastic cover and after 3 weeks they were completely uncovered and hardened to green house condition.

RESULTS AND DISCUSSION

In significant development it was observed that multiple shoot buds induced on cotyledonary nodal explants on MS medium supplemented with different concentration of BAP and KIN singly and in combination with IAA. Multiple shoot initiation took 8-10 days from date of inoculation. Multiple shoots was induced MS

Table 1: Effect of cytokinin (BAP and KN) alone and combined with IAA on multiple shoot bud regeneration from cotyledonary node explants of *Operculina turpethum*.

Hormone	Concentration	% of response	No.of shoots /explants mean ± SD	Shoot length (cm) mean ± SD
BAP	0.5	40	3.00±0.63	4.88±0.39
	1.0	50	3.37±0.86	5.08±0.28
	1.5	30	2.80±0.74	4.69±0.35
	2.0	20	2.00±0.63	3.92±0.09
	2.5	10	1.30±0.45	3.25±0.25
KIN	0.5	60	9.80±0.40	7.36±0.31
	1.0	80	11.90±0.70	8.14±0.20
	1.5	90	12.70±0.86	8.35±0.22
	2.0	70	11.70±0.90	7.90±0.38
	2.5	70	10.90±0.83	7.06±0.14
KIN + IAA	1.5+0.1	80	15.70±0.45	9.25±0.25
	1.5+0.2	90	16.10±0.94	9.75±0.25
	1.5+0.3	80	15.20±0.40	8.65±0.45
	1.5+0.4	70	13.90±0.53	7.90±0.37
	1.5+0.5	60	12.50±0.50	7.20±0.24

Table 2: Effect of different concentration of IBA on root induction of shoots derived from cotyledonary node explants of *Operculina turpethum*

Hormone	Concentration	% of response	No.of roots / shoots mean ± SD	Shoot length(cm) mean ± SD
HMS	-	30	2.40±0.66	2.29±0.36
IBA	0.1	50	4.40±0.80	4.25±0.46
	0.3	70	5.80±0.60	4.74±0.43
	0.5	90	7.30±0.64	5.05±0.30
	0.7	80	6.30±0.64	3.40±0.32
	0.9	30	3.50±0.80	2.45±0.24

Fig. 1: Regeneration of plantlets from the cotyledonary nodal explants.
a. Natural Habitats of *Operculina turpethum*, b. *In vitro* seed germination using Half strength MS medium, c. multiple shoot buds developed from cotyledonary node explants on MS medium supplemented with KIN + IAA (1.5+0.2 mg/l), d. *in vitro* raised single shoots were rooted in MS medium supplemented with IBA (0.5mg/l).

medium supplemented with different concentration of KIN and BAP (0.5-2.5mg/l). Frequently, number of multiple shoots was more on MS medium containing 1.5 mg/l KIN tested (Table 1). In tobacco, kinetin proved 30,000 times more effective in multiplication of shoots than BAP [16]. In higher concentration of BAP callus was formed at the base of young shoots, this kind of results has been achieved in *Vigna radiata, Canavalia virosa*, *Widalia calendulaceae* [17, 18, 19]. Effect of supplementing auxin IAA at 0.1-0.5mg/l to cytokinin (BAP and KIN) containing medium was studied. High frequency and maximum number of multiple shoots were elicited on MS medium containing 1.5 mg/l KIN + 0.2mg/l IAA (Table 1, Fig. 1). At higher concentration of IAA, callus was produced in the base of the explants and similar result was obtained in *Gymnema sylvestre* [20]. Periodical subculture (16-21 days interval) is used for getting increased number of shoots from the cotyledonary node and to avoid the basal callus induction.

In vitro raised shoots were excised from the shoot clumps and cultured MS medium containing different concentration of IBA (0.1-0.9mg/l) for root initiation. In IBA (0.5 mg/l) proved most suitable for root induction (Table 2). After four weeks, the *in vitro* raised plantlets were transferred to greenhouse for acclimatized and planted in the field successfully.

High frequency and maximum number of multiple shoots were elicited on MS medium supplemented with combination of KIN and IAA. In KIN +IAA (1.5+0.2mg/l) enhance the rate of multiplication and elongation of shoot (8-10 cm) length (Table 1).

Micropropagation from cotyledonary node play an important role in shoot production form seedling explants as they supply endogenous growth regulators to the cultures [21,24]. Rapid plant multiplication and improvement through biotechnological methods are limited for *Operculina turpethum*. An efficient reproducible *in vitro* propagation system using cotyledonary node explants has been used for development of mass propagation of this medicinally as well as endangered plant species.

CONCLUSIONS

This study conclude that the multiple shoots of *Operculina turpethum* were developed from cotyledonary nodal explant on Murashige and Skoog (1962) medium supplemented with the hormone Kinetin (KN) combined with IAA at the concentration of1.5+ 0.2 mg/l. This study was efficiently developed a standard protocol to initiate multiple shoot and root from the cotyledonary node.

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