BCSIR

Available online at www.banglajol.info

Bangladesh J. Sci. Ind. Res. **47(4)**, 433-436, 2012

BANGLADESH JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

E-mail: bjsir07@gmail.com

Plantlet regeneration from nodal explants of *Ocimum citriodorum* Vis.

B. Janarthanam[a] and E. Sumathi[b]*

[a]*Omnigreen Organic Biopark Pvt. Ltd., Plant Biotechnology Division, AGS Colony, Alwarthiru Nagar, Chennai- 600 087, India.*

[b]*Department of Biotechnology, D.G. Vaishnav College, University of Madras, Arumbakkam, Chennai-600106, Tamil Nadu, India.*

Abstract

A protocol for multiple shoot induction and plant regeneration from nodal explants of *Ocimum citriodorum* has been developed. Nodal explants inoculated on Murashige and Skoog (MS) medium supplemented with 1.0 mg/l Benzyl adenine (BAP) and 0.025 mg/l indole -3-acetic acid (IAA) showed better growth response (80%) and produced 15.2 ± 1.28 shoots per explant with an average length of 6.17 ± 0.29 cm after 35 days. Roots were induced after transfer to half strength MS medium supplemented with 0.5 mg/l Indole -3- butyric acid (IBA) produced 6.0 ± 1.0 roots with an average height of 4.9 ± 0.26 cm after 30 days. Plantlets with well developed root and shoot systems were successfully acclimated (80 %) and established in earthen pots containing mixture of soil, vermiculite and farm yard manure (1:1:1).

Keywords: *Ocimum citriodorum*; Plant regeneration; Nodal explant; Mass propagation

Introduction

Medicinal plants are an important source of compounds for the pharmaceutical industry and traditional medicine. About 80% of the population living in developing countries still use traditional medicines derived from plants for their primary health care needs (Cunningham, 1993; De Silva, 1997). The genus *Ocimum family* labiatae (lamiaceae) has long been used as a medicinal and aromatic plant in many countries like Egypt, India, Greece, Italy, Morocco and others. It contains between 50 to 150 species of herbs and shrubs from tropical and subtropical regions of Asia, Africa as well as central and South America (Shadia *et al*., 2007). In India, basil is often referred to as the "king of herbs". The plant is held sacred by Hindus all over the world as it is used for religious purposes, in addition to its great medicinal values (Banu and Bari, 2007). Eugenol is a phenolic compound and major constituent of essential oils extracted from different parts of *Ocimum* plants. The therapeutic potential of *Ocimum* plants has been used in several pharmacological studies carried out with eugenol (Prakash and Gupta, 2005)

Diverse medicinal properties of *Ocimum* plants have been used as antidiabetic, immunostimulant, antioxidant, cardioprotection, antifungal activities and the extracts are used in ayurvedic remedies for common colds, headaches, inflammation, stomach disorders, heart disease and malaria. (Mukherjee *et al*., 2006, Samson *et al*., 2007). The conventional method for propagation of *Ocimum species* is via seed. However, poor germination potential restricts its multiplication. The plant cannot be vegetatively propagated as well (Patnaik and Chand 1996).

Ocimum citriodorum (Lemon basil) is a hybrid between basil (*Ocimum basilicum*) and African basil (*Ocimum americanum)*. It is an herb grown primarily in northeastern Africa and southern Asia, for its strong lemon fragrance used in cooking and also in antioxidant tea bags. Lemon basil has stems that can grow up to 20-40 cm. It has white flowers in late summer to early fall. The leaves are similar to basil leaves, but tend to be narrower. Seeds form on the plant after flowering and dry on the plant. Lemon basil is a popular herb in Arabian, Indonesian, Laotian, Persian, Thai *(maenglak)* cuisine

In vitro plant regeneration from nodal explants has not been reported so far. The present study aims at developing a simple, rapid, economical, and high frequency regeneration protocol from nodal explants of *Ocimum citriodorum* for large scale propagation.

Material and methods

Plant material

Healthy plants of *Ocimum citriodorum* collected from Azhiyar (Coimbatore), Tamil Nadu, India and were raised in

*Corresponding author. E-mail: sumathiethiraj@yahoo.com

pots containing soil and farm yard manure (1:1) under green house conditions at the Department of Biotechnology, D.G. Vaishnav College, Chennai- 600 106.

Explant preparation and shoot regeneration

Nodal segments of *Ocimum citriodorum* were cut from processed aseptic culture. Explants were cleaned thoroughly under running tap water for 20 minutes; followed by washing with Tween 20 solution (2 drops in 100ml water) for 1 minute and then with sterile distiled water. The cleaned explants were finally treated with $HgCl_2$(0.1% w/v) for 4-5 minutes under aseptic conditions and washed 5 times with sterile distilled water to remove traces of $HgCl_2$ The process of explant preparation was done in the Laminar Flow Chamber.

After surface sterilization, explants were trimmed to 0.8 - 1.0 cm and inoculated on MS basal medium supplemented with BAP (0.25, 0.50, 1.00 and 2.00 mg/l) and IAA (0.010, 0.025, 0.050 and 0.100 mg/l) for shoot multiplication. At the end of the experiment, percentage of shooting, shoot length and the number of shoots per explant were recorded after 35 days. The proliferated shootlets (6.0 cm in length) were excised from cultures and transferred to half strength MS medium supplemented with IBA (0.1, 0.2, 0.5, 1.0 and 2.0 mg/l) for *in vitro* rooting. Root number and length were recorded after 30 days. Well developed plantlets were rinsed thoroughly with sterile water to remove residuals and potted with a mixture of red soil, vermiculite and farm yard manure (1:1:1) covered with transparent polyethylene bags to ensure high humidity. After 15 days, the fully acclimatized plantlets were transplanted to plastic pots (80 mm diameter).

Culture medium and conditions

MS basal medium supplemented with 3% sucrose was used for all *in vitro* culture studies. The pH of the medium was adjusted to 5.6 $\pm$ 0.2 prior to adding 0.9 % agar, and autoclaved at 121 °C for 15 minutes. Cultures were maintained at 25 $\pm$ 1°C under 16h photoperiod with a photosynthetic photon flux density (PPFD) of 50 µmol $m^{-2}s^{-2}$ provided by cool white fluorescent tubes (Phillips, India) and with 60 -65 % relative humidity. The plant growth regulators (PGRs) were filter-sterilized using 0.2µm filter (Minisart®, Sartorius,VivaScience AG, Hannover, Germany) prior to adding to culture media.

Statistical Analysis

Each experiment was repeated three times and each treatment had six replicates. The data were analysed using analysis of variance (ANOVA) and the means were compared using the Duncan's multiple range test (DMRT) at 5% level of significance ($p < 0.05$).

Results and discussion

Multiple shoots developed from shoot tip explants cultured on MS supplemented with BAP (0.25 - 2.5 mg/l) and IAA

Table I. Effect of different concentration of cytokinin (BAP) and auxin (IAA) on *in vitro* shoot multiplication from nodal explants of *O. citriodorum*

PGR Conc. (mg/l)		% response	No of shoots	Shoot length (cm)
BAP	IAA			
0.25	0.010	20.00 ±0.00^{b}	2.00 ±1.00^{abc}	1.80 ±0.10^{b}
	0.025	41.67 ±5.77^{f}	2.00 ±0.00^{abc}	1.97 ±0.06^{bc}
	0.050	55.00 ±5.00^{g}	2.67 ±0.58^{abcd}	2.10 ±0.17^{c}
	0.100	23.33 ±2.89^{b}	1.33 ±0.58^{ab}	1.40 ±0.17^{a}
0.50	0.010	10.00 ±0.00^{a}	1.00 ±0.00^{a}	1.97 ±0.06^{bc}
	0.025	25.00 ±5.00^{bc}	3.00 ± 1.73^{bcd}	2.40 ±0.10^{d}
	0.050	31.67 ±2.89^{cd}	4.00 ±1.00^{d}	2.87 ±0.12^{ef}
	0.100	18.33 ±5.77^{b}	1.67 ±1.15^{ab}	1.43 ±0.06^{a}
1.00	0.010	35.00 ±5.00^{def}	3.67 ±1.53^{cd}	2.93 ±0.06^{f}
	0.025	80.00 ±5.00^{h}	15.2 ±1.28^{e}	6.17 ±0.29^{g}
	0.050	40.00 ±5.00^{ef}	4.00 ±1.00^{d}	2.90 ±0.10^{ef}
	0.100	35.00 ±5.00^{def}	3.67 ±0.58^{cd}	2.67 ±0.29
2.00	0.010	18.33 ±5.77^{b}	2.00 ±0.00^{abc}	2.03 ±0.15^{bc}
	0.025	33.33 ±2.89^{de}	1.00 ±0.00a	1.83 ±0.06^{bc}
	0.050	35.00 ±5.00^{def}	2.00 ±1.00^{abc}	1.97 ±0.15^{bc}
	0.100	20.00 ±0.00^{b}	2.00 ±1.00^{abc}	1.43 ±0.06^{a}

Explants were cultured on MS basal media supplemented with BA and IAA. Data were recorded after 35 days of culture. Results represent mean ± SD of six replicated experiments. Values denoted by different letters differ significantly at $p<0.05$ level.

Fig. 1. A-H Regeneration of multiple shoots from nodal explants of *Ocimum citriodorum*

A& B) Nodal explants inoculated on MS medium supplemented with 1.0 mg/l BAP and 0.025 mg/l IAA. C) Initiation of shoot from nodal explants after three weeks of culture D & E) Proliferation of multiple shoots from internodal explants at 35 days of cultured on MS medium containing 1.0 mg/l BAP and 0.025 mg/l IAA. F) Healthy *in vitro* shootlets inoculated on half strength MS medium containing 0.5 mg/l IBA G) A well established plant H) Well established and hardened *in vitro* plants successfully transferred to the paper cups. I) Hardened plants transferred to external environment condition showing luxurious growth

(0.010 - 0.10 mg/l). Initiation of multiple shoots in most of the treatments was observed within three weeks of culture. Higher number of multiple shoot proliferation from nodal explants was observed in MS containing BAP 1.0 mg/l and IAA 0.025 mg/l showed better growth response (80%) and produced 15.2 ± 1.28 shoots per explant with an average length of 6.17 ± 0.29 cm after 35 days. (Table I, Fig. 1A,B,C,D,E). This synergistic combination of auxin and cytokinin on organogenic differentiation has been well explained in plant tissue culture (Baskaran and Jayabalan 2005; Gururaj *et al.,* 2007; Janarthanam and Seshadri 2008; Ahmad *et al.*, 2010; Janarthanam and Sumathi, 2010). The combination of BAP at 1.0 mg/l along with addition of IAA (0.01 - 0.1 mg/l) was effective for stimulating regeneration of shootlets. However, BAP 1.0 mg/l and IAA 0.025 mg/l was found to be most effective, capable of inducing 80% response, and produced 15.2 ± 1.28 shoots per explants. At higher concentration of BAP (2.0 mg/l) and IAA (0.1 mg/l), a gradual decrease in the number of shoots per explant was recorded (Table I).

Individual shoots from a multiple shoot complex were separated after 28 days of culture and transferred to half strength MS supplemented with IBA (0.1 - 9.84mg/l). The root induction was initiated in two weeks culture time while the root system was well developed in four weeks (Fig. 1F,G).

The maximum rooting response (75%) was achieved on medium supplemented with IBA (0.5 mg/l), with an average of 6.0 ± 1.0 roots per shoot explant (Table II). In the present study root induction was obtained with lower concentration of IBA. The reports are supported with the findings of Janarthanam and Sumathi (2009) in which high rooting percentage in *Spilanthus calva* was achieved using IBA (0.5mg/l).

Eighty percent plantlet survival was seen after hardening of the regenerated *Ocimum citriodorum* in red soil, vermiculite and farmyard manure (1:1:1) for 3weeks. However, the rate decreased as some plants died over the next 4- 5 weeks after transfer to soil. It was observed that very gradual acclimati-

Table II. Effect of individual concentration of auxin (IBA) on rooting response of *O. citriodorum*

IBA (mg/l)	% response	Roots / shoot	Root length (cm)
0.1	30.00 ±5.00a	2.67 ±0.58a	3.07±0.51b
0.2	31.67 ±2.89a	2.67 ±1.15a	3.07±0.25b
0.5	75.00 ± 5.00c	6.00 ±1.00b	4.90±0.26c
1.0	45.00 ±5.00b	2.67 ±0.58a	2.30±0.30a
2.0	36.67 ±2.89a	2.00 ±1.00a	2.00±0.20a

Explants were cultured on half-strength MS media supplemented with IBA. Data were recorded after 30 days of culture. Results represent mean ± SD of six replicated experiments. Values denoted by different letters differ significantly at $p< 0.05$ level.

zation of *in vitro* grown plants to the external environment is most essential to *Ocimum citriodorum*. Seventy percent of the plants transferred to pots survived and resumed growth (Fig1. H,I).

Conclusion

In conclusion, the results showed the ability of the nodal explants to produce higher number of shootlets without any intervening callus phase, where all the plantlets were uniform in height and growth. Hence, we propose this protocol as a simple, economical, rapid and highly reproducible to obtain more plantlets within a short period of time.

References

Ahmad N, Faisal M, Anis M and Aref IM (2010), *In vitro* callus induction and Plant regeneration from leaf explants of Ruta graveolens L. *South African Journal of Botany.* **76:** 597 - 600.

Banu LA and Bari MA (2007), Protocol establishment for multiplication and regeneration of *Ocimum sanctum* Linn.: an important medicinal plant with high religious value in Bangladesh, *Journal of Plant Science* **2:** 530-537.

Baskaran P and Jayabalan N (2005), An efficient micropropagation system for *Eclipta alba* a valuable medicinal herb. *In Vitro* Cellular and Developmeny Biology - Plant **41:** 532 -539.

Cunningham AB (1993), African medicinal plants: setting priorities at the interface between conservation and primary healthcare. People and plants working paper 1, 92. UNESCO, Paris.

De Silva T (1997), Industrial utilization of medicinal plants in developing countries. In: Bodeder G, Bhat KKS, Burley J and Vantomme P [eds.], *Medicinal Plants Forest Conservation and Healthcare*. Non-Wood Forest Products No.11. FAO, Rome, Italy

Gururaj HB, Giridhar P and Ravishankar GA (2007), Micropropagation of *Tinospora cordifolia* (wild) Miers ex Hook. F & Thoms - a multipurpose medicinal plant. *Current. Science* **92:** 23 - 26.

Janarthanam B and Seshadri S (2008). *In vitro* manipulations *of Rosa bourboniana*. Acta Horticulturae **769:** 357-370.

Janarthanam B and Sumathi E (2010), *In vitro* plant regeneration from shoot tip explants of *Exacum travancoricum* Beedi. *Journal of Plant Tissue Culture & Biotechnology* **20:** 113-118.

Janarthanam B and Sumathi E (2009), *In vitro* shoot regeneration from nodal explants of *Spilanthes calva. Journal of Tropical Medicinal Plants.* **10:** 209 - 212.

Mukherjee PK, Maitib K, Mukherjee K and Houghton PJ (2006), Leads from Indian medicinal plants with hypoglycemic potentials. *Journal of Ethnopharmacology* **106:** 1-28.

Murashige T and Skoog F (1962), A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, **15:** 473-497.

Patnaik J and Chand PK (1996), *In vitro* propagation of the medicinal herbs *Ocimum americanum* L. syn. O. canum Sim. (Hoary basil) and O. tenuiflorum L. (holy basil), Plant Cell Report **15:** 846 - 850.

Prakash P and Gupta N (2005), Therapeutic uses of *Ocimum sanctum* Linn (Tulsi) with a note jon eugenol and its pharmacological actions: A short review. *Indian J Physiol Pharmocol,* **49**(2): 125-131.

Samson J, Sheeladevi R and Ravindran R (2007), Oxidative stress in brain and atioxidant activity of Ocimum sanctum in noise exposure. Neurotoxicology **28**: 679-685.

Shadia E, Aziz AE Elsayed A and Omer Sabra AS (2007). Chemical Composition of *Ocimum americanum* Essential Oil and Its Biological Effects Against, *Agrotis ipsilon*, (Lepidoptera: Noctuidae) Research *Journal of Agriculture and Biological Sciences,* **3**: 740 - 747.

Received: 04 September 2011; Revised:25 November 2012; Accepted:26 February 2012 .