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Full Length Research Paper

In vitro multiplication of *Ocimum gratissimum* L. through direct regeneration

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The objective of this study was to develop a rapid system for regeneration of the important medicinal plant, *Ocimum gratissimum* L, from nodal explant. Single node explants were inoculated on basal MS (Murashige and Skoog, 1962) medium containing 3% (w/v) sucrose, supplemented with different concentrations and combinations of 6-benzylaminopurine (BAP), kinetin (KN), indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA) for direct plant regeneration. Maximum numbers of shoot (14.3±1.5) were observed on the medium containing 0.5 mg/l BAP and 0.25 mg/l IAA after four weeks of culture. Regenerated shoots were separated and rooted on same half strength MS medium supplemented with 0.5 mg/l of IAA alone for three weeks. Well-developed complete plantlets were transferred on to specially made plastic cup containing soilrite. Acclimatized plantlets were successfully grown in garden soil.

Key words: *Ocimum gratissimum* L., node explants, multiplication, direct regeneration, plantlets.

INTRODUCTION

Ocimum gratissimum L. is a valuable multi purpose medicinal plant which belongs to the family Lamiaceae, and is distributed in tropical and warm temperature regions. *O. gratissimum* is commonly used in the treatment of various diseases e.g., upper respiratory tract infections, diarrhea, headache, fever, ophthalmic and skin diseases and pneumonia. Extracts of the plant contains antimicrobial activity (Adebolu et al., 2005), antibacterial activity (Nakamura et al., 1999), antifungal activity (Lemos et al., 2005), antimalarial activity (Ezekwesili et al., 2003), and antiprotozoal activity (Holetz1 et al., 2003). The active compounds present as volatile aromatic oil from the leaves consist mainly of thymol (32 – 65%) and eugenol (Pino et al., 1996). It also contains xanthones, terpenes and lactone (Ijaduola et al., 1980).

Generally, *O. gratissimum* is conventionally propagated by the seed germination and stem cutting. The problem associated with conventional method of propagation is that very poor germination rate of seeds (<10%) and cuttings takes 28 days for rooting (Sulistiarini, 1999). So far, there is no report on *in vitro* method of propagation for this plant in order to improve its cultivation. Therefore this first report on *in vitro* multiplication of *O. gratissimum* through direct plant regeneration technique offers an effective alternative method of propagation for this important multipurpose medicinal plant.

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MATERIALS AND METHODS

Explants were collected from two years old-field grown mature plants, cut into 1.0 to 2.0 cm nodal segments and used for induction of multiple shoots. Explants were washed thoroughly under running tap water for 30 min and treated with a surfactant, Tween 20 (10 drops per 100ml of sterilized distilled water). Later these explants were surface sterilized with 0.1% mercuric chloride (w/v) for 5 min and washed thrice using sterilized distilled water. Under aseptic conditions, explants were inoculated on basal MS (Murashige and Skoog, 1962) medium containing 3% (w/v) sucrose, supplemented with different concentrations and combinations of 6-benzylaminopurine (BA: 0.0, 0.25, 0.5 1.0 and 2.0 mg/l), kinetin (KN: 0.0, 0.25, 0.5 1.0 and 2.0 mg/l), indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA) (0.0, 0.10, 0.25, 0.5 and 1.0 mg/l) for direct plant regeneration and root induction. The pH was adjusted to 5.8 prior to the addition of 0.8% agar and autoclaved at 121°C (1.06 kg/cm^2) for 15 min. Cultures were then incubated at 26±2°C

Table 1. Effects of different concentrations of BAP, KN alone and in combination with IBA or IAA in MS medium for multiple shoot induction from node explants of *Ocimum gratissimum* L.

Growth regulators (mg/l)	% of explant showing response	No. of shoots (cm)	Average length of shoots	Degree of root response
BAP				
0.25	60.0	10.8±1.6	2.1±0.60	_
0.50	95.0	12.0±0.5	2.4±0.29	_
1.00	90.0	11.9±0.5	2.3±0.34	_
2.00	70.0	11.0±0.4	2.0±0.30	_
KN				
0.25	50.0	6.2±1.6	1.3±0.10	_
0.50	65.0	7.0±0.4	1.5±0.13	_
1.00	70.0	8.0±0.5	2.0±0.32	_
2.00	70.0	7.9±0.5	1.8±0.21	_
BAP+IAA				
0.5+0.10	95.0	13.7±2.7	6.3±0.301	*
0.5+0.25	100.0	14.3±1.5	6.8±0.236	**
0.5+0.50	90.0	13.2±0.6	6.6±0.249	***
0.5+1.00	90.0	13.9±2.9	5.8±0.369	**
BAP+IBA				
0.5+0.10	80.0	11.0±0.5	2.7±0.38	*
0.5+0.25	90.0	12.2±0.6	3.1±0.52	**
0.5+0.50	85.0	12.0±0.7	3.4±0.46	**
0.5+1.00	85.0	11.9±0.4	2.5±0.28	**

15 explants and culture were maintained in each treatment and data (SE) were recorded up to seven weeks of culture.
* Rooting response

Table 2. Effects of different concentrations of IBA and IAA in MS medium for root induction from shoots of *Ocimum gratissimum* L.

Growth regulators (mg/l)	% of rooting response	No. of roots	Average length roots (cm)
IAA			
0.25	95.0	1.5±0.09	3.2±0.30
0.50	100.0	2.5±0.31	3.6±0.62
1.00	100.0	2.0±0.29	3.5±0.27
2.00	95.0	1.8±0.15	3.1±0.50
IBA			
0.25	90.0	1.2±0.06	1.7±0.32
0.50	95.0	1.5±0.10	2.4±0.29
1.00	95.0	1.6±0.19	3.1±0.42
2.00	90.0	2.0±0.28	2.5±0.26

15 culture were maintained in each treatment and data (SE) were recorded up to four weeks of culture.

with a 16-h photoperiod by cool white fluorescent tubes (Das et al., 1996) and 70-75% relative humidity (Mukherjee et al., 1991).

For root induction, separated shoots were transferred to half strength MS basal medium supplemented with different concentrations of IAA and/or IBA (0.0, 0.25, 0.50 and 1.0 mg/l) and 2% (w/v) sucrose. Rooted plantlets were thoroughly washed to remove the adhering gel and planted specially made plastic cup containing soilrite and kept in the greenhouse for acclimatization. Twenty cultures were used per treatment and each experiment was repeated at least three times. Percentage of success was scored four weeks after culture. Data collected were statistically analyzed and results presented in the tables.

RESULTS AND DISCUSSION

Preliminary studies proved that nodal explants culture in

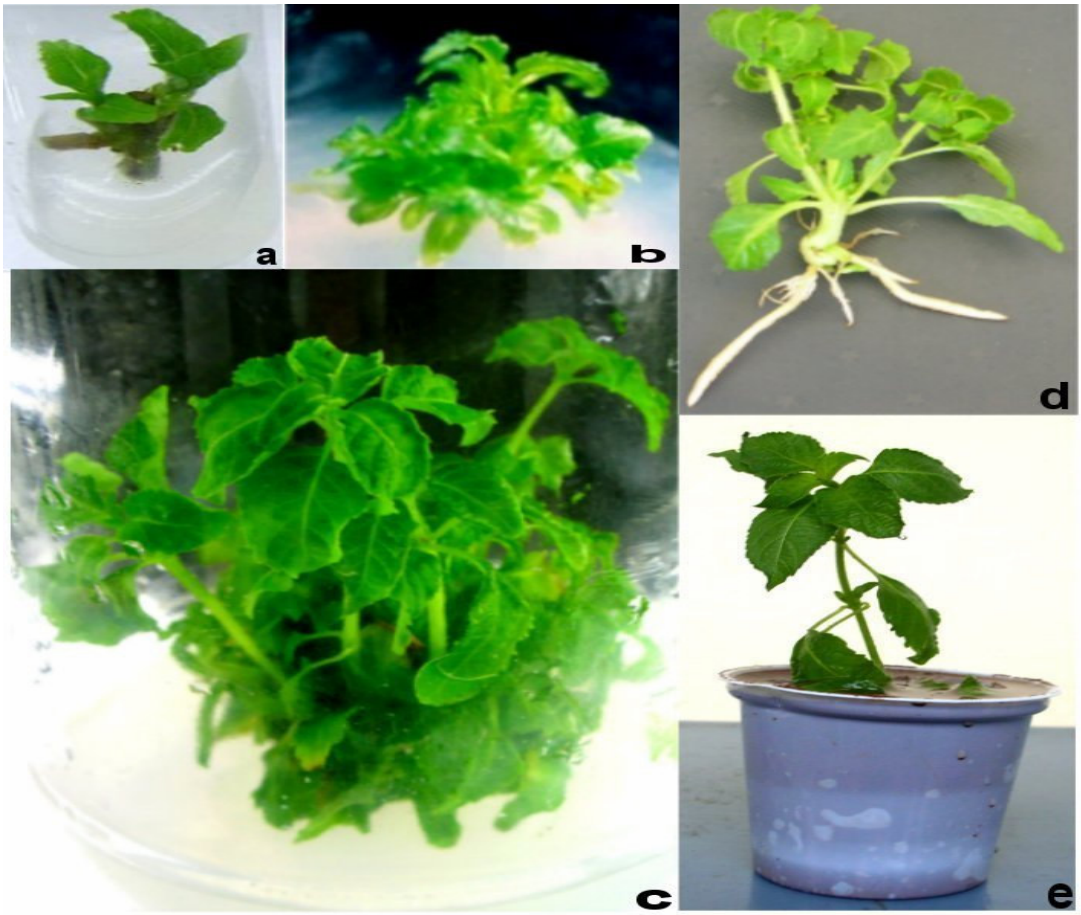

Figure 1. *In vitro* multiplication of *Ocimum gratissimum* through direct regeneration. a) Shoot induction from node explant in MS+BAP (0.5 mg/l); b) Initiation of multi shoots in MS + BAP (0.5 mg/l) and IAA (0.5 mg/l); c) Establishment of multi shoots; d) Well-developed root system and complete plant; e) Plant acclimatized to the green house.

MS medium individually supplemented with both BAP and KN showed remarkable response. Among cytokinins, 0.5 mg/l BAP responds well compare to KN in medium for shoot proliferation (see Table 1). In order to evaluate the synergistic effect of BAP with IAA and IBA for direct plant regeneration, IAA combinations responded well compare to IBA. The maximum induction of multiple shoots (14.3±1.5) was achieved from medium supplemented with 0.5 mg/l BAP and 0.25 mg/l IAA, 2 to 3 weeks after incubation, with an average shoot length of 6.8 cm (Figures 1a-c). Among the concentrations tested, the best response was noticed with 0.5 mg/l BAP and 0.25 mg/l IAA. Normally, other species like *O. basilicum* shows good response towards plant regeneration in MS medium in the presence of BAP combined with auxins as reported by various authors (Dode et al., 2003; Begum et al., 2002; Phippen and Simon 2000; Sahoo et al., 1997). In the present study, the axillay buds on further multiplication survived well and developed as four individual plants. In contrast to propagation studies, the callus induction on MS medium supplemented with 2,4-dichlorophenoxy acetic acid (2,4-D) is very low and callus formation was observed only after 3 weeks of incubation. The effect of kinetin is very less compare to BAP in shoot elongation. After 3 to 4 weeks, when regenerated shoots reached a length of more than 5 cm, they were separated and planted on half strength MS basal medium with and without IAA. In cultures, where the shoots were inoculated on auxins free basal medium, no root formation was observed. Whereas root primordia emerged from the shoot base on first week of culture on auxin-supplemented medium. Maximum number root (2.5±0.31) were produced from IAA at 0.5 mg/l (Figure 1d).

For acclimatization, plantlets were removed from rooting medium after three weeks of incubation and transferred to plastic pots containing autoclaved soil trite covered with perforated polythene bags to maintain humidity and were kept under culture room conditions for one week. After three weeks, polythene bags were removed and transferred to green house and placed under shade until growth was observed. Then they were

planted under normal garden conditions (Figure 1e).

Direct shoot multiplication is preferred for generating true-to-type plants than callus regeneration. This study supports the rapid multiplication of this useful medicinal plant by *in vitro* conditions. This report provides a simple protocol for the micropropagation of *O. gratissimum*. Shoots can be easily derived from node cultures on BAP-containing medium and subsequently rooted on IAA-containing medium. The efficiency of the system could be improved to give rise to more shoot proliferation. This approach offers a means for producing identical plantlets from node explant of *O. gratissimum*.

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REFERENCE

Adebolu TT, Oladimeji S A, 2005, Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhoea causing bacteria in southwestern Nigeria. Afr J. of Biotechnol Vol. 4 (7), pp. 682-684.

Begum F, Amin N, Azad MAK, 2002. *In vitro* rapid clonal propagation of *Ocimum basilicum* L. Plant Tissue Culture, 12, 27-35.

Das S, Jha TB, Jha S (1996). *In vitro* propagation of cashew nut. Plant Cell Reports, 15: 615-619.

Dode LC, Bobrowski VL, Braga EJB, Seixas FK, Schunch W, 2003. *In vitro* propagation of *Ocimum basilicum* L. Maringa, 25, 435-437.

Ezekwesili CN, Obiora KA, Ugwu OP, (2004). Evaluation of Anti-Diarrhoeal Property of Crude Aqueous Extract of *Ocimum gratissimum* L. (Labiatae) In Rats. Biokemistri 16(2):122-131.

Holetz1 FB, Nakamura TU, Filho BPD, Cortez DAG, Díaz JAM, Nakamura CV, (2003). Effect of Essential Oil of Ocimum Herpetomonas samuelpessoai, Acta Protozool. (2003) 42: 269 - 276

Ijaduola G, Anyiwo I, Thomas C,(1980) *Ocimum gratissimum* and blood coagulation. J. of research in Ethinomedian 1:19 – 21.

Lemos JA, Passos X S, Fernandes OFL, Paula JR, Ferri PH, Souza LKH, Lemos AA, Silva MRR, (2005). Antifungal activity from *Ocimum gratissimum* L. towards Cryptococcus neoformans, Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol. 100(1): 55-58.

Mukherjee A, Unnikrishnan M, Nair NG (1991). Growth and morphogenesis of immature embryos of Sweet Potato (*Ipomea batata* L.) In vitro. Plant Cell Tissue and Organ Cult. 26: 97-99.

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

Nakamura CV, Nakamura TU, Bando E, Melo AFN, Cortez DAG, Filho BPD. (1999) Antibacterial Activity of *Ocimum gratissimum* L. Essential Oil, Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol. 94(5): 675-678.

Pino JA, Rosado A, Fuestes V, (1996) Composition of the essential oils from the leaves and lowers of *Ocimum gratissimum* L. grown in Cuba. J. of Essential Oil Res. 8:139 – 141.

Phippen, WB, Simon JE,(2000). Shoot regeneration of young leaf explants from basil (*Ocimum basilicum* L.), In Vitro Cell. Dev. Biol. Plant, Largo, 36, 250-254.

Sahoo Y, Remien YN, Yao RS,(1997). *In vitro* clonal propagation of an aromatic medicinal herb *Ocimum basilicum* L. (sweet basil) by axillary shoot proliferation. In vitro Cell. Devel. Biol. Plant, Largo, 33, 293-296.

Sulistiarini D, (1999). *Ocimum gratissimum* L. In Oyen, LPA, Nguyen Xuan Dung (Eds.): Plant Resour of South-East Asia. No. 19: Essential-oils plants. Prosea Foundation, Bogor, Indonesia. Pp. 140-142.