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In vitro regeneration of *Ocimum minimum* L. - The smallest basil plant

Nand Kishor Sharma*, Vandana, Mithilesh Kumar

Department of Agricultural Biotechnology and Molecular Biology, Faculty of Basic Sciences and Humanities, Rajendra Agricultural University, Pusa, P.O. Box 848125, Samastipur, Bihar, (INDIA)

E-mail: nkbiotech@yahoo.co.in

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ABSTRACT

The purpose of present study was to develop a micropropagation protocol of the important medicinal plant, *Ocimum minimum* L. from nodal stem explant. Nodal stem explants were inoculated on basal MS medium supplemented with different concentrations of 6-benzylaminopurine (BAP), kinetin (KN) and combination of BAP with indole-3-acetic acid (IAA) for plant regeneration and 2,4-D were also used for callus induction. Maximum numbers of shoot (9.75±0.53) with highest length of shoots (5.95±0.08cm) were observed on the MS medium containing 1.0 mg/l BAP after 30-35 days of culture. Regenerated shoots were separated and rooted on half strength MS medium supplemented with 1.0 mg/l of IBA alone for 25-28 days. Well-developed rooted plantlets were transferred to specially made plastic cup containing a mixture of garden soil, farm yard manure and sand (1:1:1) in controlled environment for longer duration for achieving high survival percentage (70-80%) for this plant. Acclimatized plantlets were successfully grown in garden soil. Thus, first time a comprehensive micropropagation protocol was developed for *Ocimum minimum* L. and it can be used for commercial propagation and in genetic improvement studies.

KEYWORDS

Ocimum minimum L.;
Nodal stem;
Micropropagation.

INTRODUCTION

Basil belongs to the genus *Ocimum* L. and also known as sacred herb of India. The genus has its great importance in production of essential oils and aroma compounds[18]. It is a valuable multipurpose medicinal plant which belongs to the family Lamiaceae and is distributed in tropical and subtropical regions of Asia, Africa, Central and South America[14]. It is group of a culinary herbs and attractive fragrant ornamental plants[12]. Important essential oil constituents reported from *Ocimum* spp. include linalool, linayl acetate, geranio, citral, camphor, eugenol, methyl eugenol, methyl chavicol, methyl cinnamate, thymol and safrol. These having immense value in the perfumery and cosmetic industries[2].

O. minimum L. is the smallest basil amongst basil plant species and known as bush basil. It is used in the treatment of problems related to the digestive system[7]. The leaves and flowering tops are antispasmodic, aromatic, carminative, digestive, galactogogue, stomachic and tonic[9]. Externally, they are used to treat acne, loss

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of smell, insect stings, snake bites and skin infections. The leaves can be harvested throughout the growing season and are used fresh or dried. Extracts from the plant are bactericidal and are also effective against internal parasites.

Generally, Basil is conventionally cultivated by the seed germination, but the major problems related with conventional method of propagation is that very reduced germination rate of seeds (< 10%)[21]. Many *in vitro* studies have been also reported in Lamiaceae species, including *Ocimum basilicum* L.[1,4,16,17], *Ocimum gratissimum*[10], *Ocimum sanctum*[3,4,19]; but to our knowledge there are no previous reports on micropropagation of *Ocimum minimum* through nodal stem explants. In this work, a complete plantlet regeneration system was developed for this important medicinal herb.

MATERIALS AND METHODS

Plant materials

Nodal stems from one year old plants of *Ocimum minimum* L. were used in the present study. The explants were collected from Hi-Tech Horticulture garden of Rajendra Agricultural University, Pusa, India, washed thoroughly under running tap water for 20 min and then treated with 5% Teepol for 15 min, followed by 4 rinses in sterile distilled water. The nodal stems were then surface-sterilized with 70% alcohol for 1 min followed by immersion in 0.1% (w/v) aqueous mercuric chloride ($HgCl_2$) solution for 3-4 min and finally rinsed 3 or 4 times with autoclaved sterile distilled water inside a laminar air flow cabinet. The surface sterilized shoots were cut into 0.5 - 1.0 cm long stems, each containing a single node.

Culture media and conditions

The cut stems were then cultured individually on MS medium supplemented with different concentrations of BAP, KN and in combination (BAP+KN). For regeneration of complete plantlets, isolated shoots were rooted on half strength of MS medium supplemented with IBA singly. Both proliferation and rooting media contained 3% sucrose and gelled with 0.8% agar (Hi-Media, India). The pH was adjusted to 5.8 with 1N NaOH or 1N HCl. All media were steam sterilized under 1.1 kg/cm^2 pressure at 121°C. The inoculated culture tubes were transferred to the tissue culture chamber having controlled environment conditions such as temperature 25 ° ± 2°C and relative humidity (RH) 50 to 80 %. The continuous light of about 2 kilo lux was maintained through tube lights.

Shoot multiplication

For shoot multiplication, the nodal stem explants were placed on MS medium supplemented with various concentrations of 6-benzyladenine (BA, 0.5-2.0 mg/l), kinetin (KN, 0.5-2.0 mg/l) as sole growth regulator and in combinations (BA 0.5 mg/l + IAA 0.10-1.0 mg/l).

When these shoots attained length of 3-4 cm, they were excised, cut into single segments and further multiplied for four or five subcultures at 15-20 days intervals on MS media containing BA (1.0 mg/l).

Rooting of shoots

For root induction, multiple shoots grown *in vitro* were cultured on half MS basal medium supplemented with different concentrations of IBA (0.5-2.0 mg/l). The number of shoots that produced roots was documented after 20 days of incubation.

Acclimatization

Complete rooted plantlets were removed from the culture medium and the roots were washed gently under running tap water to remove agar. The plantlets were transferred to plastic pots of 5 cm diameter containing a mixture of garden soil, farm yard manure and sand (1:1:1) in controlled environment for longer time. After acclimatization the plantlets were transferred to a greenhouse with simulated habitat and the survival percentages were recorded.

Statistical analysis

The experiments followed a completely randomized design and were done at least four times. Ten explants per replicate were used in each treatment. Data were analyzed by one way ANOVA and the mean values from treatments were compared using Duncan's multiple range test at $p = 0.05$ with SPSS ver. 16. The results were expressed as means±SE of four experiments.

RESULTS AND DISCUSSION

MS basal[13] medium (control) gave no response; the callus initiation was started from the basal and cut portion of the explants. The 2.0 mg/l 2,4-D treatment yielded the maximum callogenesis (90.52%). The callus was friable in nature having yellowish white color (Figure 1a&1b). The 2,4-D was most effective in inducing callus formation. In other work, BAP, KN and BAP+IAA also showed the callus development but the frequency of callus formation was very low (TABLE 1).

TABLE 1 : Effect of different concentrations (mg/l) of 2,4-D, BAP, KN and BAP+IAA supplemented with liquid MS medium on regeneration of shoots from nodal stem explants of *O. minimum* L. Values represent means±SE. Means followed by the same letter in columns are not significantly different by the Duncan's multiple range test at 5 % probability level.

2,4-D	BAP	KN	IAA	Callogenesis (%)	Callus colour[Nature] /Growth rate	Caulogenesis (%)	Number of shoots [explant^{-1}]	Shoot length cm
0.5	-	-	-	60.83	WY^F/++	-	-	-
1.0	-	-	-	81.54	WY^F/++	-	-	-
1.5	-	-	-	83.89	YW^F/+++	-	-	-
2.0	-	-	-	90.52	YW^F/++++	-	-	-
-	0.5	-	-	15.25	WY^F/+	82.63	6.74±0.41^{f}	4.91±0.08^{f}
-	1.0	-	-	20.85	WY^F/+	90.86	9.75±0.53^{g}	5.95±0.08^{g}
-	1.5	-	-	22.50	WY^F/+	82.63	6.66±0.23^{f}	4.46±0.12^{e}
-	2.0	-	-	21.25	WY^F/+	81.94	5.83±0.16^{e}	3.76±0.05^{bc}
-	-	0.5	-	10.50	WY^F/+	77.23	2.75±0.08^{ab}	3.75±0.04^{bc}
-	-	1.0	-	12.25	WY^F/+	86.95	4.08±0.15^{cd}	4.91±0.04^{f}
-	-	1.5	-	15.25	WY^F/+	74.10	2.75±0.08^{ab}	4.29±0.12^{de}
-	-	2.0	-	16.50	WY^F/+	70.83	2.33±0.13^{a}	3.54±0.08^{b}
-	0.5	-	0.10	18.25	WY^F/+	83.60	3.08±0.15^{b}	2.78±0.10^{a}
-	0.5	-	0.25	20.75	WY^F/+	88.64	5.66±0.19^{e}	4.80±0.12^{f}
-	0.5	-	0.50	23.50	YW^F/+	86.10	4.41±0.08^{d}	4.04±0.04^{cd}
-	0.5	-	1.00	22.50	WY^F/+	78.81	3.41±0.16^{bc}	3.49±0.20^{b}

Colour : Y = Yellow, W = White/ Nature : F = Friable / Growth rate : + = poor, ++ = good, +++ = very good, ++++ = excellent

In multiple shoot proliferation nodal stem explants showed better response than other explants viz, leaves and inter nodes for this reason all experiments were carried out using nodal stems. The nodal stem explants under direct organogenesis on MS medium supplemented with various concentration and combinations of BAP, KN and combination of BAP and IAA were studied (TABLE 1). The 1.0 mg/l BAP treatment yielded the maximum regeneration (90.86%) and the maximum number of multiple shoots (9.75±0.53) with the maximum shoots length (5.95±0.08cm). *O. minimum* did not showed good response towards plant regeneration in MS medium in the presence of BAP combined with auxins (IAA) as reported by various authors in *O. basilicum*[4,8,15,17]. Between cytokinins, BAP was more responsive than KN (TABLE 1) and these findings were consonance with earlier reports in *O. killimandscharicum*[16], in *O. gratissimum*, *O. viridae*, *O. americanum*, and *O. sanctum*[1] and in *Coscinium fenestratun*[11].

After 30-35 days, the well-developed shoots were transferred to half strength MS medium supplemented with IBA singly. In different concentration of IBA tested (TABLE 2), 1.0 mg/l IBA in half strength MS medium was found to be most appropriate for rooting (Figure 1g). The stimulatory effect of IBA on root formation has been reported in many other medicinal plant species, including *Ocimum basilicum*[17], *Picrorhia kurrooa*[6], *Murraya koenigii*[5] and *W. somnifera*[20]. Root development was slow at higher IBA concentrations and these results are in accord with findings from previous studies[17].

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TABLE 2 : Effect of IBA concentration on root induction from *in vitro* raised shoots of *O. minimum* L. after 25-30 days of culture. Values represent means±SE. Means followed by the same letter in columns are not significantly different by the Duncan's multiple range test at 5 % probability level.

IBA (mg/l)	Rooting (%)	Number of roots [shoot^{-1}]	Root length [cm]
0.5	87.50	2.66±0.19^{b}	1.95±0.05^{a}
1.0	97.50	4.24±0.08^{c}	2.95±0.04^{b}
1.5	85.00	2.75±0.16^{b}	2.24±0.08^{a}
2.0	80.00	1.83±0.09^{a}	2.22±0.19^{a}

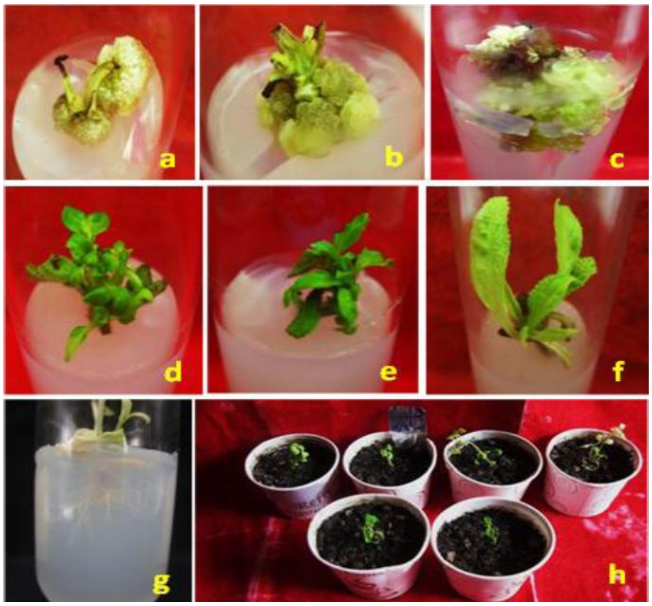

Figure 1 : Micropropagation of *Ocimum minimum* L. (a-b) Callus induction from cutting edges of nodal stem explants on MS basal supplemented with various conc. of 2,4-D (0.5-2.0 mg/l). (c-d) Shoot proliferation from nodal stem explants on MS medium supplemented with BAP (1.0 mg/l) after 30 days of culture. (e-f) Shoot proliferation from nodal explants on MS medium supplemented with constant concentration of BAP (0.5 mg/l) and varying concentrations of IAA (0.1, 0.25, 0.5 and 1.0 mg/l) after 25-45 days of culture. (g) Formation of roots from regenerated shoots cultured on ½ MS Medium supplemented with IBA (1.0 mg/l). (h) *O. minimum* plantlets developed *in vitro*, transferred to plastic cups containing garden soil, farm yard manure and sand (1:1:1).

CONCLUSION

The present study offers a micropropagation protocol for development of highest multiple shoots of *Ocimum minimum* L. Presence of 2,4-D in the media causes callusing of explants. MS basal medium with lower concentration of BAP (1.0 mg/l) showed the best effect on its shoots proliferation in terms of its height and morphology. Hence, a reliable micropropagation protocol for this species from nodal stem explants has been developed, which can ensure large scale propagation, as well as its *ex situ* conservation of this important aromatic and medicinal herb.

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