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Original Article

In vitro multiple shoot induction through axillary bud of *Ocimum basilicum* L. an important medicinal plant

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Abstract

Micropropogation of *Ocimum basilicum* L. a medicinal plant known for its traditional and folk remedy to treat various ailments, has been carried out by using axillary explants on Murashige & Skoog's medium. Nodal explants produced proliferation of multiple shoots on the medium containing 0.5 mgl^{-1} BAP with 0.5 mgl^{-1} IAA. The elongated shoots were separated and cultured for root induction. Rooting of *in vitro* raised shoots were best induced on ½ strength MS medium supplemented with 1.5 mgl^{-1} IBA with highest percentage of shoot regenerating roots (89 %). The well rooted plantlets were acclimatized and successfully established in the natural condition with 90% survival.

Keywords: *Ocimum basilicum,* Medicinal plants, Regeneration, Nodal segments, Shoot tip

Introduction

Ocimum basilicum L. (Sweet basil) is a small perennial, culinary herb tropically growing shrub of Asian origin (Dhar, 2002). It is widely cultivated for the production of essential oils, and also marketed as an herb, either fresh, dried, or frozen (Putievsky *et al*., 1999). The essential oil of sweet basil possesses antifungal, insect-repelling and toxic activities (Reuveni *et al*. 1984; Dube *et al*. 1989; Werner *et al*., 1995). Chiang *et al.,* 2005 has also reported antiviral activities. The leaves and flowers of sweet basil are traditionally used as antispasmodic, aromatic, carminative, digestive, galactogogue, stomachic, and tonic agents (Lust, 1983; Chiej, 1984; Duke *et al*., 1985; Sahoo *et al.*, 1997; Phippen and Simon, 1998). *Ocimum basilicum* is also a globally important economic crop producing annually ca. 100 tonnes of essential oil worldwide and with a trade value as a pot herb of around US $ 15 million per year. It is also widely used in systems of indigenous medicine (Paton 1996). Usually the plant is regenerated through seeds and creeping stem nodes. But due to the indiscriminate collection of huge amount of this plant by local herbalists and Ayurvedic and Unany companies, this plant species is on the verge of extinction. Under such a situation it is important to develop techniques for rapid mass propagation of this species to meet up the commercial need and also for protecting the genetic erosion. *In vitro* micropropagation is an effective mean for rapid multiplication of species in which it is necessary to obtain a high progeny uniformity. Therefore, the interest in using these techniques for rapid and large-scale propagation of medicinal and aromatic plants has been significantly increased (Sahoo *et al.*, 1997).

In vitro micropropagation technique has been proved to be employed in propagation of many of medicinal plant species (Sivakumar and Krishnamurthy 2000, Selvakumar *et al*., 2001, Wawrosch *et al*., 2001, Das and Handique 2002, Kalidass *et al.,* 2008, Kalidass and Mohan 2009). Many *in vitro* studies have been conducted on Lamiaceae species, including the *Ocimun* genus, using different explants, like nodal segments (Shahzad and Siddiqui, 2000; Begun *et al.*, 2002), leaf explants (Phippen and Simon, 2000), young inflorescence (Singh and Sehgal, 1999) and axillary buds (Begum *et al.*, 2002).

Materials and Methods

Nodal segments from natural population of *Ocimum basilicum* were collected and used in the present study (Fig 1a). Nodal segments with a single axillary bud were used as explants. The explants were washed under running tap water, pre-soaked in liquid detergent for about 20 – 30 min, and surface sterilized using 70% (v/v) ethanol for 1 min and 0.1% (w/v) mercuric chloride for 2-5 min. It is then

washed with sterile double distilled water. The surface sterilized explants were sized to 1cm length contain a single node with an axillary bud. The explants were inoculated vertically on the culture medium. Shoot proliferation and adventitious shoot regeneration were achieved on Murashige & Skoog's (1962) basal medium supplemented with different concentration and combination of BAP, IAA & NAA (Table 2). The regenerated shoots were excised aseptically with the help of sterile scalpel under laminar air flow cabinet. The shoots were then inoculated on half strength of MS medium with different concentrations and combinations of IBA (indole 3-butyric acid) and NAA (napththaleneacetic acid) for root induction. Both proliferation and rooting media contained 3% sucrose and gelled with 6 % agar (Hi-Media, India). The pH was adjusted to 5.7±0.1. All media were steam sterilized under 1.1 kg/cm^2 pressure at 121°C. Cultures were grown at 25±1 °C under 16 h photoperiod with a light intensity of 2000 – 3000 lux. The well rooted plantlets were gently removed from the culture tubes without any damage, washed to remove agar and media adhered to the roots and transplanted to plastic pots filled with soil and compost (1:1) for hardening. The plantlets were kept in a polychamber at 90% relative humidity, 30±1°C under a 24h photoperiod for acclimation. Established plants were transplanted in earthen pots under natural conditions and the survival rate was recorded.

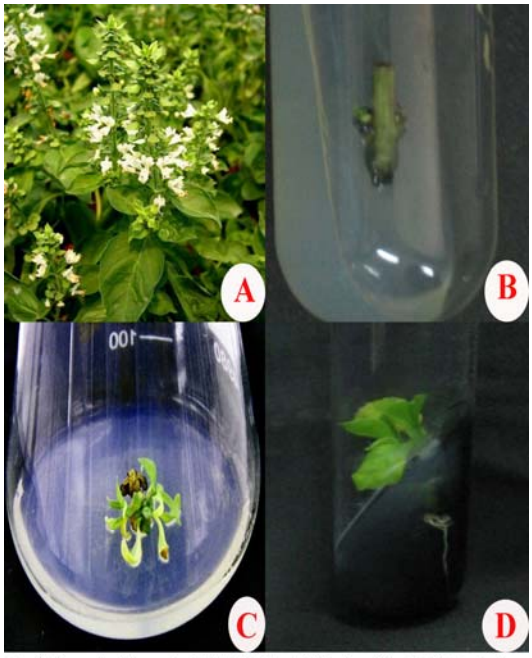
Results and Discussion

In multiple shoot proliferation nodular explants response better than other explants viz, leaves and inter nodes for this reason all experiments were carried out using nodal segments. Similar finding of auxiliary buds proliferation have also been reported in many medicinal plants (Anand and Jeyachandran 2004; Hassan and Ray 2004; Kalidass and Mohan 2009). The nodal explants under direct organogenesis on MS supplemented with various concentration and combinations of BAP, NAA and IAA were studied (Table 1). The best response with maximum shoot elongation was obtained using 1.0 mgl^{-1} BAP in combination with 0.5 mgl^{-1} IAA after four weeks of culture. Our results also show that 1.0 mgl^{-1} BAP with 0.5 mgl^{-1} IAA promotes formation of high multiple shoots (Fig 1c). *O. basilicum* shows good response towards plant regeneration in MS medium in the presence of BAP combined with auxins as reported by various authors (Sahoo et al., 1997; Begum et al., 2002; Phippen and Simon 2000 & Dode et al., 2003). The role of BAP and IAA in shoot formation has also been recorded in other medicinal plants (Arockiasamy et al., 2002; Marta *et al*., 2009 and Taware *et al*., 2010).

Table 1. Effect of different concentrations and combinations of growth regulators on MS medium for the adventitious shoot regeneration from the nodal explants of *Ocimum basilicum.*

S. No	Growth regulators (mg/l)			Shooting response (%)	Mean No. of shoots (Mean ± SE)	Mean length of shoots (cm) (Mean ± SE)
	BAP	IAA	NAA			
1	0.5	0.0	0.0	30	8.6 ±0.11	1.8± 0.21
2	1.0	0.0	0.0	42	7.9± 0.07	1.5± 0.14
3	1.5	0.0	0.0	46	9.8± 0.12	2.3± 0.17
4	2.0	0.0	0.0	28	6.2±0.08	6.6± 0.22
5	2.5	0.0	0.0	31	6.4±0.16	2.5± 0.16
6	3.0	0.0	0.0	24	7.3± 0.24	5.9± 0.26
7	0.5	0.5	0.0	71	12.5± 0.11	5.3± 0.10
8	1.0	0.5	0.0	82	23.8± 0.23	6.8± 0.14
9	1.5	0.5	0.0	64	13.8± 0.07	3.5± 0.13
10	2.0	0.5	0.0	48	12.9± 0.09	2.4± 0.17
11	2.5	0.5	0.0	56	12.8± 0.06	1.9± 0.09
12	3.0	0.5	0.0	62	14.6± 0.16	1.6± 0.11
13	0.5	0.0	0.5	52	21.6± 0.24	4.9± 0.21
14	1.0	0.0	0.5	46	12.3± 0.09	3.2± 0.14
15	1.5	0.0	0.5	49	13.5± 0.06	2.6± 0.18
16	2.0	0.0	0.5	40	10.7± 0.12	2.5± 0.29
17	2.5	0.0	0.5	44	11.6± 0.23	2.2± 0.26
18	3.0	0.0	0.5	36	7.3± 0.16	1.9± 0.18

After four weeks, the well developed shoots were transferred to half strength MS medium supplemented with IBA singly and in combination with NAA (Table 2). In different concentration of IBA tested, 1.5 mgl^{-1} IBA in half strength MS was found to be most suitable for root induction (Fig 1d). The supplementation of auxin either singly or in combination was also reported in many plant species (Gopi et al. 2006; Baksha *et al*., 2007; Kalidass *et al*., 2008; Kalidass and Mohan, 2009). However, the addition of IBA also favored rooting in other medicinal plants like *P. kurrooa* (Chandra *et al*., 2006), *S. cordifolia* (Sivanesan and Jeong, 2007a), *P. indicum* (Sivanesan and Jeong, 2007b), and *W. somnifera* (Sivanesan, 2007).



A - *Ocimum basilicum* L. habit, B - Shoot initiation, C - Multiple shoots, D - Rooting of *Ocimum basilicum* L.

For acclimatization, plantlets were removed from rooting medium after three weeks of incubation and transferred to plastic pots containing autoclaved soil rite covered with perforated polythene bags to maintain humidity and were kept under culture room conditions for one week. Then they were planted under normal garden conditions. After hardening the growth rate of the plantlets was slow initially and increased gradually. New leaves emerged from the hardened plantlets after three weeks. Most of the plantlets (90 %) survived after hardening.

Table 2: Effect of different concentration of half strength of MS medium on root induction of *in vitro* shoots.

S. No	Growth regulators (mg/l)		Rooting response (%)
	IBA	NAA	
1	0.5	0.0	56.25±1.41
2	0.0	0.5	45.71±1.72
3	0.1	0.0	47.14±2.56
4	0.5	0.0	62.50±3.10
5	1.0	0.0	81.25±0.83
6	**1.5**	**0.0**	**89.00±0.73**
7	2.0	0.0	60.00±1.22
8	1.0	0.5	51.25±2.03
9	1.5	0.5	38.88±2.02
10	2.0	0.5	00.00±0.00

About 90% of the regenerated plantlets could tolerate and survive under *ex vitro* environment or field conditions. A number of plantlets were lost due to damping off and necrosis during acclimatization in *ex vitro* condition. Loss of regenerants due to such symptoms was also observed in *Eucalyptus tereticornis* (Gill et al. 1993), *Solanum nigrum* (Ara et al. 1993), *Rauvolfia serpentine* (Ilahi 1993) and *Rosa damascena* (Kumar et al. 1995). 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. basilicum*. The shoots can be easily derived from node cultures on BAP and IAA containing medium and subsequently rooted on IBA containing medium. This protocol could be utilized for conservation and clonal propagation of this economically important medicinal plant.

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