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Enhanced Axillary Shoot Proliferation in *Ocimum sanctum* Linn. via Shoot Tip Culture Using Various Concentrations of BAP

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ABSTRACT

Preliminary studies on micropropagation of holy basil (*Ocimum sanctum* L.), an Indian medicinal herb, have been conducted via enhanced axillary branching on Murashige and Skoog (MS) medium utilizing in vitro raised shoot tips. Of various levels of BAP tested (0.5 to 5.0µM) maximum number of enhanced axillary shoots was observed at the concentration of 1µM. Rooting of the main elongated primary explant was also observed on same medium. Isolated axillary shoots also behaved in a similar manner on the said medium. 80% plantlets when transplanted to vermiculite + peat moss mixture survived under laboratory conditions.

Keywords: Shoot tip, enhanced axillary branching, micropropagation, rooting, *Ocimum sanctum*, BAP

Abbreviation: BAP- 6-Benzyl Amino Purine, MS- Murashige and Skoog medium, TDZ-Thidiazuron.

INTRODUCTION

Ocimum sanctum (Syn *O. tenuiflorum*) also known as holy basil is a well known aromatic plant in the family Lamiacae. It is an erect sweet scented pubescent herb, 30-100 cm in height. This herb is found through out India and cultivated mostly for ceremonial purposes. The plant is held sacred by Hindus all over the world besides having great medicinal value. The leaves on steam distillation yield a bright yellow volatile oil possessing a pleasant odour, characteristic of the plant, with an appreciable note of cloves. This oil possesses antibacterial and insecticidal properties and inhibits *in vitro* growth of *Mycobacterium tuberculosis* and *Micrococcus pyogenes* var. *aureus*. Besides oil, leaves also contain ascorbic acid and carotene. The juice of leaves has diaphoretic, antiperiodic, stimulating and expectorant properties. It is also used in catarrh, bronchitis and is considered to have adaptogenic properties. It is anti-inflammatory, analgesic and a tonic for central nervous system.

The conventional method for propagation of *Ocimum sanctum* is only via seed. However, poor germination potential restricts its multiplication and does not retain the pure line. Unfortunately, this plant cannot be vegetatively propagated (Pattnaik and Chand, 1996). *In vitro* micropropagation is an effective means for rapid multiplication of species in which conventional methods have limitations (Arora and

Bhojwani, 1989; Kartha, 1985; Nehra and Kartha, 1994; Sen and Sharma, 1991; Sudha and Seeni, 1994). Although many *in vitro* studies have been conducted on other members of the family Lamiaceae (Sahoo et al., 1997; Sen and Sharma, 1991; Sunnichan and Shivanna, 1998; Khosla, 1995), there are few reports on this plant in which micropropagation has been achieved (Pattanaik and Chand, 1996; Singh and Sehgal, 1999). Present study is an attempt to use shoot tips for micropropagation through enhanced axillary branching of the plants under the conditions prevailing in the valley.

MATERIAL AND METHODS

Seeds of *O. sanctum* were thoroughly washed using lab wash, surface sterilized in aqueous 0.1% $HgCl_2$ for 10-15 minutes and finally sterilant was removed by repeatedly washing in sterile double distilled water. These seeds were then germinated on autoclaved moistened filter paper in petriplates. Shoot tips excised from these seedlings were cultured individually under aseptic conditions on Murashige and Skoog's (1962) medium containing 3% sucrose as a carbon source and 0.8% agar for gelling. This served as basal medium. The medium was supplemented with a range of 0.5 to 5.0 µM concentrations of 6- benzyl amino purine (BAP). The pH of the medium was adjusted between 5.2 to 5.8 using 0.1N NaOH or 1% HCl before autoclaving. The medium was dispensed into culture vials which were plugged with non absorbent cotton and sterilized by autoclaving at a temperature of 121°C and 15lb pressure for 20 min. Each experiment was repeated at least twice and observations were recorded at weekly intervals. Mean ± SD was calculated for number of shoots/ roots formed and length of shoots/ roots observed.

RESULTS AND DISCUSSION

The shoot tips failed to show any response and yellowing of explants was observed on MS basal medium after 3 weeks time. Such results have also been observed by Singh and Sehgal (1999) using young inflorescence in *O. sanctum* meaning thereby that endogenous levels of hormone present in the explants are not sufficient enough to initiate and sustain their growth on the basal medium which otherwise when enriched with different concentrations of cytokinin, BAP (0.5 to 5.0 µM) proved effective to show the results which are summarized in Table 1.

Mild callus formation was recorded at the base of the shoot tip explant followed by its elongation and axillary shoot formation from the tiny nodal buds of the elongated main shoot tip and thereafter regeneration of thin and long adventitious roots was also noticed from the main explant after 6 to 7 weeks of inoculation by using 0.5 µM BAP. Most of the early reports pertaining to organogenesis in members of the family lamiaceae have also suggested the effective role of BAP in the shoot initiation, either individually or in combination with other growth regulators (Singh and Sehgal, 1999; Begum *et al*., 2002).

Table 1: Response of in vitro raised shoot tips (primary explants) and isolated axillary shoots of primary cultures of O. *sanctum* to different concentrations of BAP.*

BAP (µM)	Response	Cultures forming shoots/ roots (%response)	No. of shoots formed ± SD	Shoot length ± SD (cm)	No. of roots formed ± SD	Root length ± SD (cm)
0.5	Mild callus formation at the base of the explants followed by its elongation and subsequent enhanced axillary shoots formation. The cultures also formed thin and long adventitious roots from the main explants.	90	2.4±0.5	2.5±0.5	2.5±0.4	3.8±0.8
1.0	-do-	90	11.6±1.6	14.8±0.8	14.5±1.9	3.6±0.5
1.5	-do-	90	4.4±0.8	2.1±0.8	7.2±0.8	2.6±0.4
2.0	-do-	80	2.1±0.5	1.6±0.1	2.4±0.8	1.8±0.3
2.5	Mild callus formation at base	-	-	-	-	-
3.0	Browning of explants	-	-	-	-	-
3.5	-	-	-	-	-	-
4.0	-	-	-	-	-	-
4.5 5.0	-	-	-	-	-	-
	-	-	-	-	-	-

Data scored after 8 Weeks of culture period: 10 replicates/ treatment, the results are mean of two repeated experiments.

Various levels of BAP tested (0.5 – 5.0µM) MS+BAP 1µM proved to be the most effective for enhanced axillary branching, as the length and number of shoots and roots per explant were maximal (Fig 1&2) compared to its lower concentration (0.5µM). Similarly, on increasing the concentration of BAP above 1µM (1.5 & 2.0µM), the length as well as number of axillary shoots and roots again got reduced drastically (Fig 3).

Fig1.

Fig2.

Fig3.

Fig4.

Fig5

Fig. 1-5 Shoot tip culture of *Ocimum Sanctum*

Fig 1.Enhanced axillary shoot formation on BAP (1µM).

Fig 2. Reduced axillary shoot formation on BAP (1.5 µM).

Fig 3. Complete plantlet formation with roots and axillary shoots from the primary explant on BAP(1µM).

Fig 4. Complete plantlet formation with roots and axillary shoots from the isolated axillary shoot on the same medium containing BAP (1µM).

Fig 5.Survived plantlet after transplanting to plastic cups filled with vermiculite and peatmoss (1: 1).

Further increase in the strength of BAP concentration (2.5, 3.0, 3.5, 4.0, 4.5 & 5.0µM) resulted in only mild callus formation at the base of the explant with 2.5µM and rest of the indicated concentrations favoured no signs of growth and thus eventually explants showed necrosis and browning after 4 weeks. Similar results have also been observed in leaf explants of *Pogostemon cablin* (Misra, 1996), axillary shoot bud explants of *Ocimum sanctum* (Pattnaik and Chand, 1996) and young inflorescence of *O. sanctum* (Singh and Sehgal, 1999). Begum *et al.,* 2002 also achieved maximum number of shoots from nodal and shoot tip explant of *Ocimum basilicum* on lower concentration of BAP (0.8µM) while as Siddique and Anis (2007) achieved rapid micropropagation of *O.basilicum* shoot tip explants in liquid MS medium supplemented with different concentrations of TDZ.

The axillary shoots produced from the primary cultures were then isolated and again transferred to the fresh medium of the same composition for rooting. But along with rooting, axillary shoots proliferation was recorded in the same manner as in primary cultures (Fig 4). In vitro raised plantlets were subsequently transferred individually to plastic cups filled with vermiculite and peat moss (1: 1 ratio). These plantlets resumed normal growth and 80% survived in pots under laboratory conditions (Fig 5).

CONCLUSIONS

In the present preliminary tissue culture study of *O. sanctum* it was observed that the high frequency of enhanced axillary shoots can be accomplished on MS + BAP (1µM) using proper micropropagation procedure. In addition, rooting of the main shoot tip explant can be achieved on the same medium at the same time which can save the time involved in raising the plantlets. Hence the protocol is simple and quite reproducible.

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