Micropropagation of an Important Medicinal Plant Ocimum sanctum for Field Plantation

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Abstract

Ocimum sanctum known as 'Tulsi' in Hindi and 'Holy Basil' in English is an erect softy hairy aromatic herb or under shrub found in India. It belongs to herbal family Lamiaceae. This plant has described as Dashmani Shwaaharni (antiasthmatic) and Antikaphic drugs (kaphaghna) in India's medicinal history. An efficient method for rapid propagation has been developed by using young shoot of Ocimum sanctum. Young shoot cultured in Murashige and Skoog, (MS) medium containing different growth regulatory components like as indole-3 acetic acid (IAA), α-naphtholene acetic acid (NAA), 6-benzyl aminopurine (BAP), and sucrose. The callus further elongated by transferring it in fresh media after a specific time interval. The maximum number of shoots was achieved with medium containing BAP. Rooting of shoot was achieved by using MS medium supplemented with 2.0 mg/l IAA and 3% sucrose. Well developed plantlets transferred in polycup containing sterile soil with compost material and finally well established in the field with 60-70% survival rate.
KEYWORDS: Ocimum sanctum, Lamiaceae, MS medium, callus.

INTRODUCTION

Plants are the most important sources of medicine. In present era the large number of drugs in use derived from plants (*Satyavati etal, 1976*). According to a survey (1993) of World Health Organization (WHO), the practitioners of trasditional system of medicine treat about 80% of patients in India, 85% in Burma and 90% in Bangaladesh (*Nadkararni etal, 1976, Siddiqui etal,* 1993). In traditional systems of medicine the Indian medicinal plants have been used in successful management of various disease conditions like bronchial asthma, chronic fever, cold, cough, malaria, dysentery, convulsions, diabetes, diarrhea, arthritis, emetic syndrome, skin diseases, insect bite etc. and in treatment of gastric, hepatic, cardiovascular & immunological disorders (*P. Prakash etal, 2005*). The writings indicate that therapeutic use of plants is as old as 4000–5000 B.C. and Chinese used first the natural herbal preparations as medicines. In India, however, earliest references of use of plants as medicine appear in Rigveda which is said to be written between 3500–1600 B.C. Later the properties and therapeutic uses of medicinal plants were studied in detail and recorded empirically by the ancient physicians in Ayurveda (an indigenous system of medicine) which is a basic foundation of ancient medical science in India (*Sirkar etal, 1989*).

In Ayurveda Tulsi *(Ocimum sanctum L.)* has been well documented for its therapeutic potentials. It has described as Dashemani Shwasaharni (antiasthmatic) and antikaphic drugs (Kaphaghna) (*Sirkar etal, 1989*). Tulsi is known as "Queen of plants" "The mother medicine of nature". Tulsi i.e. *Ocimum sanctum* is a plant with enormous properties for curing and preventing diseases (*Vinod etal,2010*).. In India Tulsi is taken as the most important plant. *Ocimum sanctum* (Tulsi) belongs to the

family Lamiaceae. It is found in tropical and sub tropical areas of all over the world also in India. It is an erect, sweet scented herb. In Sanskrit "Tulsi" means "the incomparable one". Whole plant is used as a source of remedy (*Ahmed etal,2002*). In India two forms of Tulsi are more common - dark or *Shyama* (Krishna) Tulsi and light or Rama Tulsi. The former possesses greater medicinal value and is commonly used for worship. Various other species are also commonly found in India like *O. canum*, *O. basilicum*, O. *ilimandscharicum, O. ammericanum, O. camphora* and *O. micranthum(Bhargava etal, 1981).* Whole tulsi plant has several therapeutic properties is used as an expectorant, analgesic, anticancer, anti-asthmatic, anti-emetic, diaphoretic, anti-diabetic, anti-fertility, hepatoprotective, hypotensive, hypolipidemic etc(*Vinod etal,2010*) .

Tissue culture is an age-old practice for *in vitro* regeneration of plants, especially the medicinally valuable plant or plants that are difficult to propagate in natural environment(*Atal etal,1989*). This method is also used for studying the plant development at the molecular level thereby artificially increasing the plant output molecules(*Dhar etal,1968).* So we describe here an efficient procedure for the rapid micropropagation of *O. sanctum* through young shoot tip culture, which is often used for production of large scale plantlets (*Banerjee etal,1996*). Shoot tip culture is widely used for rapid propagation of many species due to its advantages over traditional methods (Hu and Wang, 1983). The present investigation aims to optimize the best *in vitro* culture condition and media combinations for micropropagation of given species for the sussessful field plantation (*Khanna et al, 2003*).

MATERIALS AND METHODS

Collection of explant material

Mature seeds of *O. sanctum* were collected from Pt. Jawaharlal Nehru Agriculture University., Jabalpur and cultivated in botanical garden of Rajiv Gandhi College Sherganj, Satna (M.P.). Plants with 6 to 8 leaves were selected as mother plant and observe the proper growth and development. After the 2-3 weeks of growth young shoot tip were collected from mature plants which has used as a explant material. The shoot tips transferred immediately into distilled water after collection (*Chattopadhayay etal,1993*).

Surface Sterilization

The explants were washed in running tap water for 2-3 times and transfer into 15% sodium hypochlorite for 15 minutes. The seeds were then washed in distilled water 4-5 times. Put the seeds into 70% alcohol for 1 minute. The seeds were washed with autoclaved distilled water 3-4 times. Then dip the seeds in 0.07% aqueous mercuric chloride solution for 2 minutes the rinse the seeds in autoclaved distilled water. After rinsing explant were used for culturing(*Chiang etal,2005*).

Culturing explant material

Expant material cultured on murashige and skoog (MS) medium. In this medium sucrose used for carbon requirement and agar as a solidifying agent. Media used were supplemented Kn; α- naphthalene acetic acid, NAA; BAP; IAA for the growth control. The pH of the medium was set to 5.8. About 20ml of hot medium was transfer into each culture tube and capped with polypropylene caps (*Amrani etal,2006*). Media were sterilized by autoclaving at 121°C for 15 minutes. The medium in the culture tubes was allowed to set as slants. Then inoculate the explant material aseptically. The cultures were incubated at 25 ± 2ºC and 16 hours photoperiod at 60 µmol m- 2s-1 light intensity by white fluorescent tubes, and the relative humidity was maintained at 60%. After 25 days for response of the explants.

Subsequently, percentage of response, number and length of shoot, number of leaves, and root number and length were also recorded.

Shoot Proliferation and Multiplication

The axillary buds of culture explants were transfer in MS medium supplemented with various concentrations of cytokinins (BAP) and auxins (IAA) either individually or in combinations for shoot bud initiation and shoot multiplication, and sub-cultured at 4 weeks interval.

In Vitro Rooting

For root induction, shoot tips with 3 or 4 fully expanded leaves from plants grown in vitro were cultured into MS medium supplemented with different concentrations of α-Napthaleneacetic acid (NAA) (0.5–2.0 mg/l) or IBA(0.5–2.0 mg/l). Root development has been observed after 18 days of incubation.

Acclimatization and Transfer of *in vitro*-raised Plantlets to Soil

Plantlets with healthy roots were selected for hardening. The plantlets were removed from the culture tubes with the help of forceps and were rinsed carefully with tap water for removing the agar medium. The plantlets were then transplanted into polycup containing different mixtures of soil and compost. The polycup were covered with ventilated polythene bags for about 2-3 weeks and were carefully sprayed with water and shifted to the glass house for hardening of plantlets. The optimum temperatures of the glass house at the time of transplantation were 16-25°C.. The relative humidity of the glass house was around 75%. The plantlets were watered daily. After time interval of four weeks plantlets transferred in the field.

RESULT AND DISCUSSION

In the present work, young shoot tip of cultivated mother plant of tulsi were used as the explant material. Serious problem of contamination was occurring during callus development which can be overcome by taking extra care (*Sen etal,1993*). In this study surface sterilization of explant material has been done by providing proper pretreatment. $HgCl_2$ based surface sterilization shows excellent results by reducing any chance of contamination (*Soumen etal,2010*). Ocimum plant has a large no of secondary metabolites with affect the proper growth of invitro culture and cause browning of culture. To overcume this probem antioxidant were added in the medium. The best results has been recorded in MS medium containing 5.52µM BAP and 0.60 µM IAA after 20 days incubation

Dode et al (2003) had also successfully reported micropropagation of *O. basilicum* using *in vitro* geminated plants. Subculturing of callus onto fresh medium containing the same concentrations of growth regulators resulted in the emergence of shoot buds. Multiple shoot buds were initiated on the callus cultured in MS medium supplemented with both cytokinins (BAP and Kn) and auxins (IAA and NAA) singly and in combination (*Kiran etal, 2011, Chopra etal,1993*).The manipulation of plant growth regulators is essential to optimize the induction of callus (Lim et al 2009). In our work Best results of shoot bud development has been observed in MS medium supplemented with 2.65µM Kn and 12.60µM NAA. Medium having BAP and IAA show lower frequency of shoot development. The superiority of Kn over BAP with respect to shoot bud initiation and subsequent proliferation of shoots from axillary buds has been reported in earlier studies (*Kiran etal, 2011*).

Induction of roots and establishment of plantlets

Multiple roots were developed by using MS medium supplemented with 25µM NAA and 02.52µM Kn. Rooting was very poor in medium containing IAA and BAP. The roots developed in the cultured shoots were mostly branched and hairy. It was observed that higher concentrations of NAA inhibited root formation, when combined

with Kn, the presence of NAA (auxin) inhibited root formation when combined with different concentrations of cytokinin (*Kiran etal, 2011*).. In our work induction of root was poor when the shoots were placed in MS basal medium. It has been found that in vitro regenerated shoots rooted best in half-strength MS medium supplemented with NAA only.

Establishment of the in vitro-raised plantlets

Plantlets when hardened in compost containing soil + cow dung (2:1) showed highest survivability (82.85%) after 4 weeks of transfer. Well developed rooted plantlets were gently removed from the culture tubes and thoroughly washed with sterile water to remove adhered agar and traces of medium to avoid contamination, and then plantlets were transferred to polycup containing soil and vermiculite (1:1). The polycup were covered with ventilated polythene bags for about 2-3 weeks and were carefully sprayed with water and shifted to the glass house for hardening of plantlets (*Parmar etal, 2009*). The optimum temperatures of the glass house at the time of transplantation were 16-25°C.. The relative humidity of the glass house was around 75%. The plantlets were watered daily. After time interval of four weeks plantlets transferred to larger potts filled with soil with organic manure for further growth. Finally the acclimated plants were shifted to field conditions, 81.13% of them having survived. The growth characteristics of plants raised in vitro did not show any significant morphological variations from those of the natural habitat.

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