In vitro Rapid Clonal Propagation of an Important Medicinal Plant of herbal origin *Ocimum americanum* for Field Plantation

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Abstract

Ocimum americanum are wild species and cultivated throughout tropical Africa and Asia. This plant is widely used for the treatment of vata, kapha, helminthiasis, anorexia, dyspepsia, pruritis, leprosy, vomiting, poison, migraine, and fever. Its seeds are good remedy for hyperdipsia, fever, migraine, and emaciation. 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), sucrose. The callus further elongated by transferring it in fresh media after an 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

Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources[1]. Many of these isolations were based on the uses of the agents in traditional medicine. The plant-based, traditional medicine systems continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care[2]. According to the World Health Organization (WHO, 1977) "a medicinal plant" is any plant, which in one or more of its organ contains substances that can be used for the therapeutic purposes or which, are precursors for the synthesis of useful drugs. The term "herbal drug" determines the part/parts of a plant (leaves, flowers, seeds, roots, barks, stems, etc.) used for preparing medicines[3]. Furthermore, WHO (2001) defines medicinal plant as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products.

Ocimum americanum Linn is commonly called as *Ocimum canum* is an important member of family Lamiaceae. It occurs wild and cultivated throughout tropical Africa and tropical Asia. *O. americanum* is an erect, much-branched, annual, aromatic herb having the height of 30-90 cm. In this plant stem and branches are quadrangular, yellowish-green and densely white-pilose mostly in the young parts but less when older [4].The roots are tap and appear in deep portion of soil. The leaves are simple, decussate and with petiolate. The length of petiole is up to 2.5 cm. The leaf-blade is lance-shaped to elliptical, measuring 2.5-5 cm x 1-2.5 cm, wedge-shaped at the base. The colour of leaves either green or purplish black depending upon light or

shaded condition. Leaves are entire in margin, acute apex, hairless and with gland-dotted on both surfaces. Verticillasters inflorescence has been observed in this plant'

Traditionally, medicinal use of Ocimum americanum has been observed in various communities by various researches. Hoary basil is widely used in folk medicine as a carminative, diaphoretic and stimulative factor to cure respiratory and hepatic infection and especially for the treatment of renal disorders [5]. Among the Santals who are the largest tribal community of India, who live mainly in the states of Jharkhand, West Bengal, Bihar, Orissa, and Assam. It has been used as a remedy for the treatment of various diseases i.e. during fever when the extremities are cold; the leaves made into a paste are applied to the finger and toe nail[6]. The same preparation of leaves is used in the treatment of parasitical diseases of the skin [7]. At Accra, the plant is used to cure dysentery [8]. For hemorrhage from the nose, the Sutos either inhale the smoke from buring the dried leaf or apply an ointment made with the powdered leaf [9].

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[10]. This method is also used for studying the plant development at the molecular level thereby artificially increasing the plant output molecules[11]. 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[12]. Shoot tip culture is widely used for rapid propagation of many species due to its advantages over traditional methods [13]. 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[14].

MATERIALS AND METHODS

Collection of explant material

Mature seeds of *O. americanum* were collected from Mohaniya Ghati Forest of Rewa District and cultivated in Herbal garden of A.K.S. University, Sherganj, Satna (M.P.). Plants with 6 to 8 leaves were selected as parental plant and observe the proper growth and development. After the 8-10 days 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[15].

Surface Sterilization

The explants were washed in running tap water for 2-3 times and transfer into 1% teepol solution for 10 minutes and rinse with DD H_2O, then placed in 70% ethanol of 60 seconds. Then dip into 1.5% of Mercuric Chloride Solution for 10-15 minutes. The seeds were then washed with autoclaved distilled water 4-5 times. After rinsing explant were used for culturing[16].

Culturing explant material

Expant material has been cultured in murashige and skoog (MS) medium. In this medium sucrose was 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[17]. 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 3-4 weeks 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[18].

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[19].

Hardening 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[20]. 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[21]. After time interval of four weeks plantlets transferred in the field[22].

RESULT AND DISCUSSION

In the present work, young shoot tip of cultivated parental plant of *O. americanum* were used as the explant material. Serious problem of contamination occurred during callus development was overcome by using ascetic and sterile culture condition. The surface sterilization of explant material has been done by using different chemical i.e. teepol, ethanol $HgCl_2$ etc. this pretreatment become very supportive for reducing any chance of microbial contamination. Different concentrations as well as ratio of harmones were used for proper growth and development of plantlets. Also some of the specific inhibitors were added for reducing chance of secondary metabolite contamination. During Ocimum generation best results has been observed after 3 week of inoculation in MS medium containing 7.34µM BAP and 0.45 µM IAA. Initially culture has been initiated in Dark followed by 10-16 hrs, photoperiod of 2000 lux cool white fluorescent light.

After successful regeneration of callus, it has been transferred for sub culturing in fresh medium containing different concentration of auxins and cytokinins hormones. Usually both of the hormones have been applied singly or in combination for optimization of culture conditions. The manipulation of plant growth regulators is essential to root and shoot induction in callus. In our work Best results of shoot bud development has been observed in MS medium supplemented with 3.08µM Kn and 10.98µM 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.

Root Induction and plantlet development

Growth of root has been induced by using MS medium supplemented with 28.37µM NAA, and 03.17µM Kn. Rooting was very poor in medium containing IAA and BAP. More branched and hairy root has been observed in plants by using optimum growth

conditions. It has been observed that higher concentrations of NAA induced the rooting when used individually as compared to combination with cytokinins. In this investigation best results of shoot has been observed with induction of root was poor when the shoots were placed in MS basal medium half-strength MS medium supplemented with NAA only.

Hardening of raised plantlets

After the complete rooting plantlets were removed from culture flask and thoroughly washed with autoclaved distilled water to remove trace amount of culture medium. Then plantlets were hardened in poly cups containing sterile soil along with vermiculite in the ratio of 3:2. The poly cups 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. For the successful growth of plantlets 16-25°C temperature and 70% relative humedity has been maintained. Highest survivability (79.68%) has been observed after 2 week of incubation. After four weeks plantlets were transferred to larger pots containing sterile soil and organic manure for further growth. Finally the acclimated plants were shifted to field conditions, 75.42% 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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