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Citation: : Komal Shekhawat, Swarnlata Kumawat, Ravi Kumar, Rekha Choudhary, Kumari Rekha, M. L. Jakhar (2020). *In vitro* root Induction in Guggul (*Commiphora wightii*) critically endangered Medicinal Plant. *Chemical Engineering*. v01i01, 158-163. <http://dx.doi.org/10.53709/CHE.2020.v01i01.022>

DOI:
<http://dx.doi.org/10.53709/CHE.2020.v01i01.022>

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Received on: September 27 2020
Revised on: November 18 2020
Accepted on: December 27 2020

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RESEARCH ARTICLE

In vitro root Induction in Guggul (*Commiphora wightii*) critically endangered Medicinal Plant

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ABSTRACT

Present investigation reflected IBA showed different morphology of roots as well as root frequency. Very thick and small roots were observed at 0.1 – 0.3 mg/l IBA, whereas thick and medium-long roots were observed at 0.4 and 0.9 mg/l IBA. Thick and profuse roots were observed at 0.5 mg/l IBA. Thick and medium-long roots were observed at 0.6 -0.8 mg/l IBA. Thick and small roots were observed at 1.0 -2.0 mg/l IBA. Root frequency ranged from 10-90 per cent. The highest roots were observed at 0.5 mg/l IBA with 90 per cent frequency.

Keywords: callus, in vitro, culture media, explants, plant, callus

INTRODUCTION

India has a treasure of well-recorded and traditionally well-practised knowledge on medicinal plants [1]. More than 6000 plants are used in our traditional, folk and herbal system of medicine [2]. India is endowed with a rich genetic resource of medicinal plants and is rightly called the “Emporium of Medicinal Plants” [3]. *Commiphora wightii* (Arnott) is a medicinally important plant that is now considered a critically endangered species of the family Burseraceae and has the chromosome number $2n = 26$ [4]. It is an important medicinal plant of the herbal heritage of India [5]. It is known by various names like guggul in Hindi, gukkulu and maishakshi in Tamil, guggulu in Sanskrit and Indian bdellium in English [6]. The genus *Commiphora* is widely distributed in tropical regions of Africa, Madagascar, Asia, Australia and the Pacific Islands (Good, 1974) [7]. In India, it is found in arid, rocky tracts of Rajasthan and Gujarat, Maharashtra and Karnataka (Kumar and Shankar, 1982) [8]. In Rajasthan, it is found in districts namely Jaisalmer, Barmer, Jodhpur, Jalore, Sirohi, Ajmer, Sikar, Churu, Jhunjhunu, Pali, Udaipur, Alwar (Sariska Tiger Reserve), Jaipur (Ramgarh, Jhalana area), Bhilwara and Rajsamand [9]. Guggul is a woody shrub with knotty, crooked, spiny brown bracties, leaves 1-3 foliate leaflets, sessile with a serrated margin [10]. Fruit is dropped red ovate with two celled stone [11]. Flowers are small, brown, pink flowers unisexual small, brownish red, the fascicles polygamous sexual distribution bisexual, female and male flowers [12]. Their 3 - 4.5 mm long, usually red white pinkish, on the flowers individually or in groups appears in the 2 or 3 Fruits are red drupe, elliptical, tapered - shaped, two cell type store, rarely four your valve voice, when ripe will be red and divides into two [13]. The ash colored bark comes off in flakes exposing the under bark which also peels off in thin papery tolls [14]. The shrub defoliates in

winter and reserves for guggul gum extraction are high during April -May [15]. This prominent species of the arid tracts of Rajasthan and Gujarat states (northwest India), *Commiphorawightii*, is now on the verge of extinction over much of its Indian range and is listed as endangered (IUCN 2010) [16]. The predominant reasons for its fast diminishing populations are over exploitation (tapping of woody shoots for its oleo-gum-resin), poor natural germination rate and slow growth rate [17]. The resin extracted from stem is considered by some to have tremendous value as cholesterol reducing agent and hence a favorite of the ayurvedic medicine industry [18-22]. This has resulted in widespread indiscriminate tapping for the resin [19]. The magnitude of the conservation problem facing *C. wightii* through this exploitation is greatly exacerbated by the fact that a plant after being tapped through deep cuttings, usually dies within two to six months of a single tapping episode [23-25]. It is not yet clear as to why plants die after tapping [26-29].

Seeds of guggul is the major propagation source in nature. In Rajasthan and nearby arid regions flowers and seeds are constantly produced by *C. wightii* except in winter season. However, the germination of seeds are poor thus large scale plantation is not possible through this natural method. Therefore, considerable efforts are still required to find out efficient *In vitro* methods for the regeneration of this critically endangered medicinal plant. The *In vitro* propagation method can be used for clonal propagation of selected germplasm, genetic improvement, production of active compound in cell culture. *In vitro* propagation in *C. wightii* has been attempted through organogenesis and somatic embryogenesis methods by various researchers.

MATERIAL AND METHODS

The present investigation entitled "Micropropagation in Guggul [*Commiphorawightii* (Arnott)] was carried out at the Department of Plant Breeding and Genetics, S. K. N. College of Agriculture, Jobner. The details of material and methods used in the present investigation are given below under separate headings:

Plant Material

The present research work was conducted on

Commiphorawightii. Nodal segments and shoot apices were used as explants and obtained from healthy trees grown in S.K.N. College of Agriculture, Jobner.

Culture Medium

All chemicals used in the present study were of analytical grade. Murashige and Skoog Medium (1962) was used throughout the course of investigation. After preparing the stock solutions of various components, the medium was prepared by mixing these stock solutions in a manner so as to maintain the final concentration of each. Stock solution of plant growth regulators were prepared by adding desired concentration of auxins and cytokinins.

Glassware

The borosilicate glass wares were used for all the experiments. Oven dried (250°C) Erlenmeyer flasks (conical flask), round bottom flask, flat bottom flasks, pipettes, Petri dishes, beaker, measuring cylinders (50 ml, 100ml, 500 ml, 1000 ml and 2000 ml) and test tubes were used for media preparation.

Autoclaving

Media were sterilized in autoclave. Distilled water, micronutrient and other stable mixtures were autoclaved. The culture media contained in glass containers sealed with cotton plugs and covered with aluminum foils were autoclaved at 15 psi. and 121°C for 15 - 40 minutes.

Sterilizing Culture Room and Transfer Area

Initially, the culture rooms were cleaned by gently washing all the floors and walls with a detergent soap followed by daily cleansing with phenyls. Transfer area was sterilized by exposure to UV light. Aseptic condition of transfer area was maintained by installing an HEPA filter ventilation unit. Laminar airflow hoods were sterilized by wiping the working surface with 95 per cent ethyl alcohol.

Explant Preparation and Sterilization

Explant was washed thoroughly in running tap water for 20 minutes, these were again

washed with liquid detergent (Rankleen) for ten minutes with vigorous shaking. After washing with detergent, explants was again washed with running tap water to remove any trace of detergent for 5 minutes. Finally explants were surface sterilized with 0.1 per cent HgCl_2 in a laminar air flow cabinet for 3 - 4 minutes.

Culture Conditions

All cultures were incubated at $25 \pm 2^\circ\text{C}$ under fluorescent light in a 14 : 10 hour's photoperiod.

Rooting

The micro propagated plantlet was subjected to different levels of auxin (IBA) for the induction of roots.

IBA (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.25, 1.50, 1.75, and 2.0 ml/l)

Hardening

After 40-50 days of culture on rooting media, the plantlets was shifted to root trainer for their hardening prior to final transfer to soil in natural conditions.

RESULTS

Root induction

Three to five centimeters long shoots were excised individually from the proliferated shoot clumps and cultured on rooting medium. In present investigation, root induction was assessed in the MS media supplemented with different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.25, 1.50, 1.75, and 2.0 mg/l) of auxin (IBA). Majority of *in vitro* shoots developed roots within 20 - 25 days of incubation.

IBA showed different morphology of roots as well as root frequency. Very thick and small roots were observed at 0.1 - 0.3 mg/l IBA, whereas, thick and medium long roots were observed at 0.4 and 0.9 mg/l IBA. Thick and profuse roots were observed at 0.5 mg/l IBA. Thick and medium long roots were observed at 0.6 -0.8 mg/l IBA. Thick and small roots were observed at 1.0 -2.0 mg/l IBA. Root frequency ranged from 10-90 per cent. Highest roots were observed at 0.5 mg/l IBA with

90 per cent frequency (Table 1. and Fig. 1.).

Hardening of Plantlets

After 40 - 50 days of culture of proliferated shoots on rooting medium (MS medium supplemented with 0.5 mg/l IBA) which resulted in the sufficient rooting in shoots and complete plants were obtained. These plantlets were shifted to root trainers cups for their hardening prior to final transfer to soil in natural condition. For hardening of plants, plantlets with newly formed roots were taken out from the culture bottles with the help of forceps with utmost care to prevent any damage to the newly formed roots and dipped in lukewarm water to remove any traces of solidified agar media. After removing media, these were dipped in 1 per cent w/v solution of bavistin to prevent ant fungal infection to newly developed plants. After bavistin treatment, the plantlets were carefully planted in root trainers containing 1:1 mixture of sterile soil and vermiculite. After planting, the plants were thoroughly watered kept in culture room for 20 days. After Primary hardening plants were kept in polyhouse for further growth to attain a size of 1.5-2.0 feet for field transfer. Guggul is slow growing plant which will take at least one year to attain the desired size. The survivability in culture room was 80 per cent and in polyhouse it was 72 per cent.

DISCUSSION

Hardening of tissue cultured plant is the most crucial step in the micropropagation. The plants produced are very soft to face ambient environmental conditions (Bhojwani and Razdan, 1992). These plants are grown under controlled conditions. It refers to acclimatizing plants from indoor temperatures to the outdoors. The hardening of *In vitro* raised plantlets is essential for better survival and successful establishment. Direct transfer of tissue culture raised plant to field is not possible due to high rate of cosseted environment with a very high humidity, varied light and temperature condition and being protected from the attack of microbial and other agents.

In the present study, after 40 - 50 days of culture of proliferated shoots on rooting medium (MS supplemented with 0.5 mg/l IBA), sufficient rooting in shoots and complete plants were

Table 1. Auxins (IBA) were added singly in the MS medium for root induction in in vitro proliferated shoots in guggul.

Concentrations (mg/l)	IBA			
	Root induction	No. of roots/plant	Root length (cm)	Root morphology
0.1	+ (20)	2.5	1.5	Very thick and small root
0.2	+ (30)	3.2	1.7	Very thick and small root
0.3	+ (40)	3.4	2.3	Very thick and small root
0.4	++ (70)	5.6	3.0	thick and medium long root
0.5	+++ (90)	6.6	4.7	Thick and profuse root
0.6	++ (80)	4.5	4.4	Thick and medium long root
0.7	+ (65)	3.8	3.7	Thick and medium long root
0.8	+ (50)	3.5	3.2	Thick and medium long root
0.9	+ (40)	3.4	3.0	Thick and medium long
1.0	+ (35)	2.5	2.3	Thick and small
1.25	+(30)	2.0	2.0	Thick and small
1.5	+ (30)	1.7	1.8	Thick and small
1.75	+(20)	1.0	1.5	Thick and small
2.0	+(10)	1.0	1.0	Thick and small



Fig. 1: Root induction in nodal segment explant on MS medium supplemented with 0.5 mg/l IBA

obtained. These plantlets were shifted to root trainer for their hardening before final transfer to soil in natural condition. For hardening of plants, plantlets with newly formed roots were taken out from the culture bottles with the help of forceps with utmost care to prevent any damage to the newly formed roots and dipped in lukewarm water to remove any traces of solidified agar media. After removing media, these were dipped in 1 per cent w/v solution of bavistin to prevent any fungal infection to newly developed plants. After bavistin treatment, the plantlets were carefully

planted in root trainers containing 1:1 mixture of sterile soil and vermiculite. After planting, the plants were thoroughly watered and kept in culture room for 10 days. Then the plants were shifted to shade house under controlled humidity, temperature conditions. In shade house plants were also watered at an interval of 2-3 days. The *Commiphora* plants are slow growing in nature which will take at least one year to attain height of 2-3 feet for field transfer. These plants showed survivability in culture room and polyhouse 80 and 72 per cent, respectively.

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