Int.J.Curr.Microbiol.App.Sci (2021) *10*(03): 1896-1902

EXCELLENT PUBLISHERS

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 10 Number 03 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1003.239

Influence of Antioxidants on the Explants of Guggul (*Commiphora wightii* (Arnott)) under *invitro* Conditions

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A B S T R A C T

Shoot apex and nodal segment explants of guggul were placed on Murashige and Skoog Medium (MS medium, 1962) supplemented with different concentration of activated charcoal (20, 40, 60, 80 and 100 mg/l) and Polyvinylpyrrolidone (2, 4, 6, 8 and 10 mg/l) for shoot bud induction. Activated charcoal (20 - 100 mg/l) was added in the basal medium with responsive level of plant growth regulators (3.0 mg/l BAP) in nodal segment, maximum number of shoot bud (1.55) was observe at 60 mg/l activated charcoal with no browning intensity in the culture medium. Maximum shoot bud proliferation in nodal segment explants followed by 6 mg/l level of polyvinylpyrrolidone. Shoot apex explant was gave minimum response in activated charcoal and polyvinylpyrrolidone.

Keywords

Guggul, Callus induction, Tissue culture, *in vitro*

Article Info

Accepted:
18 February 2021
Available Online:
10 March 2021

Introduction

India has a treasure of well recorded and traditionally well practiced 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 which is now considered as critically endangered species of the family Burseraceae and having the chromosome number 2n = 26 (Sobti and Singh, 1961) (4). It is an important medicinal plant of 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, sping brown bracties, leaves 1-3 foliate leaf lets, sessile with serrated margin (10). Fruit is drops 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, 2 - cell - type store, rarely four your valve voice, when ripe will be red and divides into two (13). The ash coloured 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), *Commiphora wightii* 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). 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 (Bhatt *et al.,* 1989, Paliwal, 2010) (20). It is not yet clear as to why plants die after tapping (21).

Seeds of guggul are 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.

Materials and Methods

The present investigation entitled "Micropropagation in Guggul (*Commiphora wightii* (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 *Commiphora wightii*. Nodal segments and shoot apexes 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.

Glass ware

The borosilicate glasswares were used for all the experiments. Oven dried (250^0C) 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 121^0C for 15 - 40 minutes.

Sterilizing culture rooms 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 $25\underline{+}2^0$C under fluorescent light in a 14: 10 hour's photoperiod.

Levels of antioxidants

Antioxidants *viz.,* activated charcoal and polyvinylpyrollidone were used to control the accumulation of phenolic compounds in the culture medium and enhance the rate of Micropropogation was worked out at most responsive level of plant growth regulators.

Activated charcoal (20, 40, 60, 80 and 100 mg/l)

Polyvinylpyrrolidone (2, 4, 6, 8 and 10 mg/l).

Results and Discussion

When different antioxidants incorporated singly in MS medium supplemented with responsive level (Nodal segment, 3.0 mg/l BAP) of plant growth regulators for shoot multiplication elicited different response for shoot bud induction because it controls the accumulation of inhibitory substances (phenolic compounds) in the growth medium.

Effect of activated charcoal

When activated charcoal (20 - 100 mg/l) was added in the basal medium with responsive level of plant growth regulators (3.0 mg/l BAP) in nodal segment, it induced shoots at all the level of activated charcoal ranging from 1.37 – 1.55 within 16 – 18 days of incubation.

Maximum number of shoot bud (1.55) was observed at 60 mg/l activated charcoal with no browning intensity in the culture medium. All other level showed low browning except 60, 80 and 100 mg/l AC (Table 1, Fig. 1).

Effect of polyvinylpyrrolidone

Addition of polyvinylpyrrolidone in culture vessels with responsive level of plant growth regulator induced shoot bud from nodal segment explants at all levels. Lower level of polyvinylpyrrolidone showed low browning in culture vessels.

Perusal of Table 1 revealed that addition of activated charcoal (60 mg/l) in the MS medium with 3.0 mg/l BAP for nodal segment explants appeared most optimum level of antioxidant for maximum shoot bud proliferation in nodal segment explants followed by 6 mg/l level of polyvinylpyrrolidone. Among both the antioxidants polyvinylpyrrolidone was less effective to proliferate shoot buds (Table 1, Fig. 2).

Phenolic compounds are released in cultures from damaged part of the explant under *in vitro* conditions. Phenolic compound inhibit shoot bud proliferation via induction in enzymatic activities, killing of the explants, darkening of tissues and culture media and formation of hard crust in the base.

The most striking effect of bud break and shoot multiplication have been found with many antioxidant (Wang *et al.,* 2002 and Ujjwala, 2006). Activated charcoal, ascorbic acid, citric acid and polyvinylpyrollidone are most commonly used antioxidants for micrpropagation.

The beneficiary effects of activated charcoal on tissue responses *in vitro* could be attributed to (a) establishing polarity by darkening the medium (Dumas and Monteuuis (1995), (b) adsorption of inhibitory substances, produced by either the media or explants (Fridborg and Eriksson, 1975, and Fridborg *et al.,* 1978), (c) adsorption of plant growth regulators and other organic compounds (Constantin *et al.,* 1977, and Weatherhead *et al.,* 1978) and (d) the release of substances naturally present in or adsorbed by activated charcoal (Johansson *et al.,* 1990). It was either beneficial or harmful effects on the culture, depending upon the medium, and tissue used under *in vitro culture* (Pan and Staden, 1999). The adsorptive capacities of activated charcoal have also been shown to affect the composition of the media in a selective manner Thiamine HCl and nicotinic acid were removed from media by activated charcoal, whereas inositol and sucrose were not and activated charcoal gave the effected on callus growth and shoot organogenesis in tobacco (Constantin *et al.,* 1977) as we find in our investigation but gave the *in vitro* generated healthy shoots. The development of an activated charcoal - free media is an alternative. Activated charcoals commonly increase the roots growth and improve the in vitro morphogenic response of tissues in several ways (Dharishini *et al.,* 2015).

In the present study antioxidants (activated charcoal, and polyvinylpyrollidone) when incorporated singly in the basal medium along with micropropagation protocol (3.0 mg/l BAP) induced multiple shoots at all the levels, however, higher shoot buds were obtained at 60 mg/l activated charcoal.

The results of present investigation for use of activated charcoal were in close agreement with effect of antioxidant on micropropagation by Wu and Xi, 2002 and Wang *et al.,* 2004 where activated charcoal was prime antioxidant used for successful shoot multiplication.

Table.1 Effect of antioxidants on *in vitro* degree of browning and culture establishment of nodal segment explant in MS medium supplemented with 3.0 mg/l BAP

Antioxidant	Concentration (mg)/l	Days taken in shoot induction	Number of shoot bud induction	Browning intensity
Activated Charcoal	20	18.20	1.38#±0.15	+
	40	17.90	1.37±0.12	+
	60	17.70	1.55±0.14	-
	80	17.20	1.43±0.13	-
	100	16.30	1.38±0.10	-
Polyvinylpyrr olidone	2	16.90	1.30±0.14	+
	4	18.40	1.36±0.13	+
	6	17.90	1.44±0.12	+
	8	17.40	1.41±0.11	-
	10	17.30	1.31±0.10	-

Fig.1 Shoot bud induction in nodal segment explant on MS medium supplemented with 3.0 mg/l BAP and 60 mg/l activated charcoal.

Fig.2 Shoot bud induction in nodal segment explant on MS medium supplemented with 3.0 mg/l BAP and 6 mg/l polyvinylpyrrolidone

Sharma *et al.,* 2012. Observed better rooting when basal medium was supplemented with activated charcoal with 1.0 mg/l IAA in *Acacia leucophloea* which was contrary to the results of present investigation in which better shoots were obtained. This difference might be due to differences in the genera.

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How to cite this article:

Shekhawat, K., S. Kumawat, R. Kumari and Jakhar, M. L. 2021. Influence of Antioxidants on the Explants of Guggul (*Commiphora wightii* (Arnott)) under *invitro* Conditions. *Int.J.Curr.Microbiol.App.Sci.* 10(03): 1896-1902.
doi: https://doi.org/10.20546/ijcmas.2021.1003.239