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Biosynthesis of guggulsterone in the callus culture of *Commiphora wightii*. Arnott. bhandari (Burseraceae)

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ABSTRACT

Commiphora wightii is a hard sample which grows very slowly in natural condition but having immense medicinal importance. The propagation of *C. wightii* through the seed and stem cutting is quite difficult because of its hard nature. Maturity period of sample is also long which unable the availability of plant and produce to industry in right time. Due to over exploitation and slow growing nature it has become endangered also. The study is devoted to ensure the availability of plant and its produce at right time in right quantity and quality through tissue culture technique. Tissue culture technique has been employed for the quick isolation of Guggulsterone which is the active constituent of *C. wightii*. Callus was induced from nodal segment, leaf and embryo as explants of *Commiphora wightii* in MS medium supplemented with 2, 4–D and Kn., individually and in combinations. Highest growth of callus (5.78 g) was recorded in the treatment MS + 2, 4–D (5 mg/l) + Kn. (0.5 mg/l). Biosynthesis of guggulsterone was observed in the callus grown in all the treatments with 2, 4–D and Kn., both individually and in combination in MS medium. Quantitative estimation of guggulsterone in cultured callus was studied using TLC and HPLC respectively. Highest guggulsterone content of 0.062% was recorded in the callus cultured in MS + 2, 4–D (5.0 mg/l) + Kn (0.5 mg/l). Callus culture technique may be an important tool to get the Guggulsterone quickly as compared to the nature where it takes around fifteen years. Good quality of Guggulsterone can also be ensured by this technique as compared to the nature. One can get the active constituent (Guggulsterone) without destroying the plant available in nature.

Key words: *Commiphora wightii*, Guggulsterone, 2, 4-D, Kinetin, HPLC.

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INTRODUCTION

Commiphora Wightii (Arnott.) Bhandari, commonly known as mukul, is an important medicinal plant of herbal heritage of India, which belongs to the family Burseraceae. In English the plant is called as Indian Bdellium Tree. However other synonyms of the plant are *C. mukul* (Arnott.); *C. roxburghii* (Stocks), Engl.; *Balsamodendron wightii,* (Arnott) and *B. roxburghii* (Stocks). It is a small tree or Shrub (Fig 1 *a*), slow growing in nature, and takes about 8 to 10 years to reach the height of 3 to 3.5 meters. The economical part of the plant is the gum resin which is generally called as guggul, an oleo- gum resin mentioned by Sushruta 3000 year ago as being a valuable drug. The oleo- gum resin commonly known as gum guggul is produced in the plant by physiological process. The active component in the gum resin is a steroid, guggulsterone.

Guggulsterone is used for the preparation of medicines to treat diseases like hypercholestronemia, inflammation, oedema, pyorrhea and arthritis [1]. *In vivo* clonal propagation of *C. wightii* is poor, inefficient and difficult to grow because of low germination percentage [2]. A plant generally takes 10-15 years to reach tapping maturity under the dry climatic conditions. Efforts to achieve increased flow of resin in the plant with the help of chemicals have not been successful. In India, Gujarat is the major supplier of the gum resin followed by Rajasthan. The present production of guggul is not sufficient to meet the annual domestic demand. Several factors viz. biotic influence, faulty tapping techniques, slow growing, poor seed set, lack of cultivation are responsible for low population status of the plant [3].

Lack of sufficient production of guggul from natural sources and destruction of plant from their natural habitat, compelled to look for alternative method using biotechnological tools for guggulsterone production under *in vitro* condition. Tissue culture technique can be used for micropropagation of the plant [4] and also production of secondary metabolite can be initiated through cell and callus culture [5]. The present study was therefore undertaken to grow callus and simultaneous production of guggulsterone through callus and quantitatively estimate its synthesis by HPLC.

EXPERIMENT

Fresh explants consisting of leaf, embryo and nodal segment of *C. wightti*, were selected for *in vitro* callus initiation. The elite plant parts consisting of leaves, nodal segments and embryos were taken as explants for *in vitro* callus initiation. The elite plant was selected among different plants collected from different locations along with their resins and grown in the green house of the department. The guggulsterone content in the oleoresin was estimated to identify the potential plant by using HPLC technique (Table-1).

MS medium [6], supplemented with 2, 4-D and kinetin individually and in combinations were found to be effective for callus induction in *C. wightii*. For callus initiation MS medium was supplemented individually with 2, 4-D (1.0 – 5.0 mg/l) and Kn. (1.0 – 5.0 mg/l) and in combination of 2, 4-D (1.0 – 5.0 mg./l) with Kn. (0.1- 0.5 mg./l). Guggulsterone content in the

callus was studied from the 12 weeks old callus grown and maintained through regular subculture in the same media composition. Gelling of media was done with tissue culture grade agar (0.9 %). P^H of the media was adjusted to 5.6 with digital P^H meter. Sterilization of the media and inoculating instruments were done at 15 lb pressure for 15 minutes.

Explants were initially washed with detergent solution Tween-20, followed by surface sterilization with 0.1 % mercuric chloride solution for 5 minutes and rinsed several times in sterile double distilled water. Embryo was isolated from specially treated matured seeds with 0.1 N HCL and soaking it in sterilized water overnight before surface sterilization. Explants from nodal segments were cut into desired sizes about 1.5 cm in length, and inoculated into the culture medium, where as other explants like leaf and embryo were taken intact for inoculation. The whole operation was done aseptically under a laminar air flow cabinet. The cultures were incubated in a culture room at temperature of 25 ^{o} C ± 2 ^{o} C and photoperiod of 16-8 hours light-dark period, under cool fluorescent light intensity of 2000-3000 lux.. Data were taken for each treatment to study the callus growth. Growth of callus was studied by taking the fresh as well as the air dry weight in an electronic balance.

Extraction of gugulsterone from the callus was done by the method given by Hung *et al*, (1996) [7] and the resin based extracts were profiled qualitatively for its constituents [8]. The extract was identified with the help of readymade TLC plate using silica gel-G as the coating substance with a solvent system of light petroleum (60 – 80 ^{0}C) and ethyl acetate in the ratio of 3:1 as the mobile phase. After complete drying of plate, identification of the spot was done by UV light at wavelength of 245 nm. High performance liquid chromatography was used for quantitative estimation of guggulsterones. HPLC was performed with a Shimadzu LC – 10A (Shimadzu Corporation, Kyoto, Japan) model equipped with a supelco pack C18 column (25 x 4.6mm.x 5 micrometer), at a flow rate of 1 ml./min, using Acetonitrile and water (65:35) as the mobile phase. Quantitative estimation of the guggulsterone was made using a standard plot, made with 10 mg of standard guggulsterone [9].

RESULTS

Callus has been initiated in MS medium supplemented with 2, 4-D and Kn individually and in combinations in *C. wightii*. However good callus initiation and growth was observed with a combine effect of 2, 4-D and Kn. Maximum 5.78 g. fresh callus biomass was obtained in the treatment MS+ 2, 4-D (5 mg/l) + kn (0.5 mg/l) (Table 2). Different plant parts like embryo, leaf and nodal segment (Fig.1 *b, c, d*) can be used as explants for the tissue culture of *C. wightii* as all of them showed callus initiation in MS medium supplemented with 2,4-D and Kn. individually and also in combinations. However callus initiation was found to be better when leaf and embryo was used as explants. It was observed that callus was initially green in color (Fig 1 *e*) but latter on turned yellowish to brownish in color with maturity. 24 week old matured callus biomass (Fig 1 *f*) maintained through regular subculture was used for the isolation of guggulsterone.

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Quantitative variation of oleo-gum resin collected from the trees, grown in different geographical locations has been seen from our study. The quality of the oleoresin is determined by the amount of guggulsteron content. Resin collected from the trees grown in Galta (Rajasthan) showed highest percentage of guggulsterone, which was found to be 3.80 % and the lowest 2.10 % guggulsterone was found in the resin collected from the tree grown in Rajasthan University campus (Table 1).

Table-1: Guggulsterone content in *C. wightii* collected from different locations.

Sl. No.	Guggul resin Samples collected from different location	Guggulsterone content in resin (in percentage)
1	Galta (Rajastahan)	3.80
2	Mangaliawas (Rajastahan)	3.10
3	Khatipura (Rajastahan)	2.50
4	University campus (Rajastahan)	2.10
5	Udaipur (Rajastahan)	3.23

Biosynthesis of guggulsterone in the callus was found in all the treatments. However guggulsterone content in callus was found to be more, when induced from the nodal segments. Callus initiated from the explants viz. embryo, leaf, and nodal segments were found to contain significant amount of guggulsterone which was initially studied with TLC, followed by HPLC using marker Guggulsterone as standard (Table 2). The quantitative estimation with HPLC, showed a maximum of 0.062 % guggulsterone which was being synthesized in the callus induced from nodal explants in the treatment MS + 2, 4-D (5.0 mg/l) + Kn. (0.5 mg/l.) (Table 2). Synthesis of Guggulsterone was also observed in the callus grown in the medium supplemented individually with 2, 4-D and to a lesser amount in Kn supplemented media. Highest 0.056 % guggulsterone content was detected in the callus grown in the MS media supplemented with 2, 4-D (3 mg/l), while the corresponding figure was 0.033 % in callus grown in MS+ Kn (5 mg/l) (Table 2).

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Table-2: Callus growth and biosynthesis of guggulsterone after 24 week of culture.

Sl. No.	Treatment (mg/l)	Fresh weight of callus (g)	Dry weight of callus (g)	% age Guggulsterone content
1	MS-basal	--	--	--
2	MS+2,4-D (1)	2.01	0.152	0.043
3	MS+2,4-D (2)	2.20	0.178	0.052
4	MS+2,4-D (3)	3.11	0.255	0.056
5	MS+2,4-D (5)	3.97	0.337	0.054
6	MS+Kn (1)	0.64	0.058	0.026
7	MS+Kn (2)	0.94	0.061	0.021
8	MS+Kn (3)	1.67	0.103	0.031
9	MS+Kn (5)	1.94	0.113	0.033
10	MS+2,4-D (1)+Kn (0.1)	3.62	0.324	0.041
11	MS+2,4-D (2)+Kn (0.2)	4.56	0.423	0.048
12	MS+2,4-D (3)+Kn (0.3)	4.10	0.381	0.056
13	MS+2,4-D (5)+Kn (0.5)	5.78	0.572	0.062

DISCUSSION

The variation in the guggulsterone content in the resin of the tree of *C. wightii* during screening clearly indicates that standard drug can be produced only from the elite plant. Seasonal variation also plays a vital role in the quality as well as quantity of oleoresin produced. Explants collected during the summer showed better proliferation in culture medium, compared to that collected and cultured during winter. Similar studies on the effect of seasonal variations, during which explants are collected and cultured under *in vitro* conditions was well documented by several workers [10] and have been reported in many trees like *Techtonas* [11], sweet gum [12], guava [13]. Plant cell culture can be exploited to produce a number of fine chemicals viz. enzymes, pigments, insecticides, stimulants, essential oils and drugs. Their production by means of cell culture has in the past years developed into an important goal in plant tissue culture research.

CONCLUSION

Several workers have reported callus induction in MS media, supplemented with 2, 4-D and Kn in combination in several woody plant species viz. *Sesamum indicum* [14], *Phoenix dactylifera* [15], *Ceratozamia hildae* [16] and *Aquilaria agallocha* [17], which supports our findings for callus induction. *In vitro* production of compounds of medicinal value by callus and suspension culture was carried out in many plant species. Aminuddin and Choudhary [18], reported production of diosgenin from the callus culture of *Dioscoresa floribunda*. Similar type of works has also been reported in the production of ajamaline from *Rauwolfia serpentine* [19], barberine from *Coptis japonica* [20] and digoxin from *Digitallis lanata* [21]. Owing to its important pharmacological properties, immense therapeutic potential and endangered in

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nature, the tissue culture technique can be used for micropropagation of the plant and at the same time standard guggulsterone can be obtained by cell and callus culture under *in vitro* condition [22].

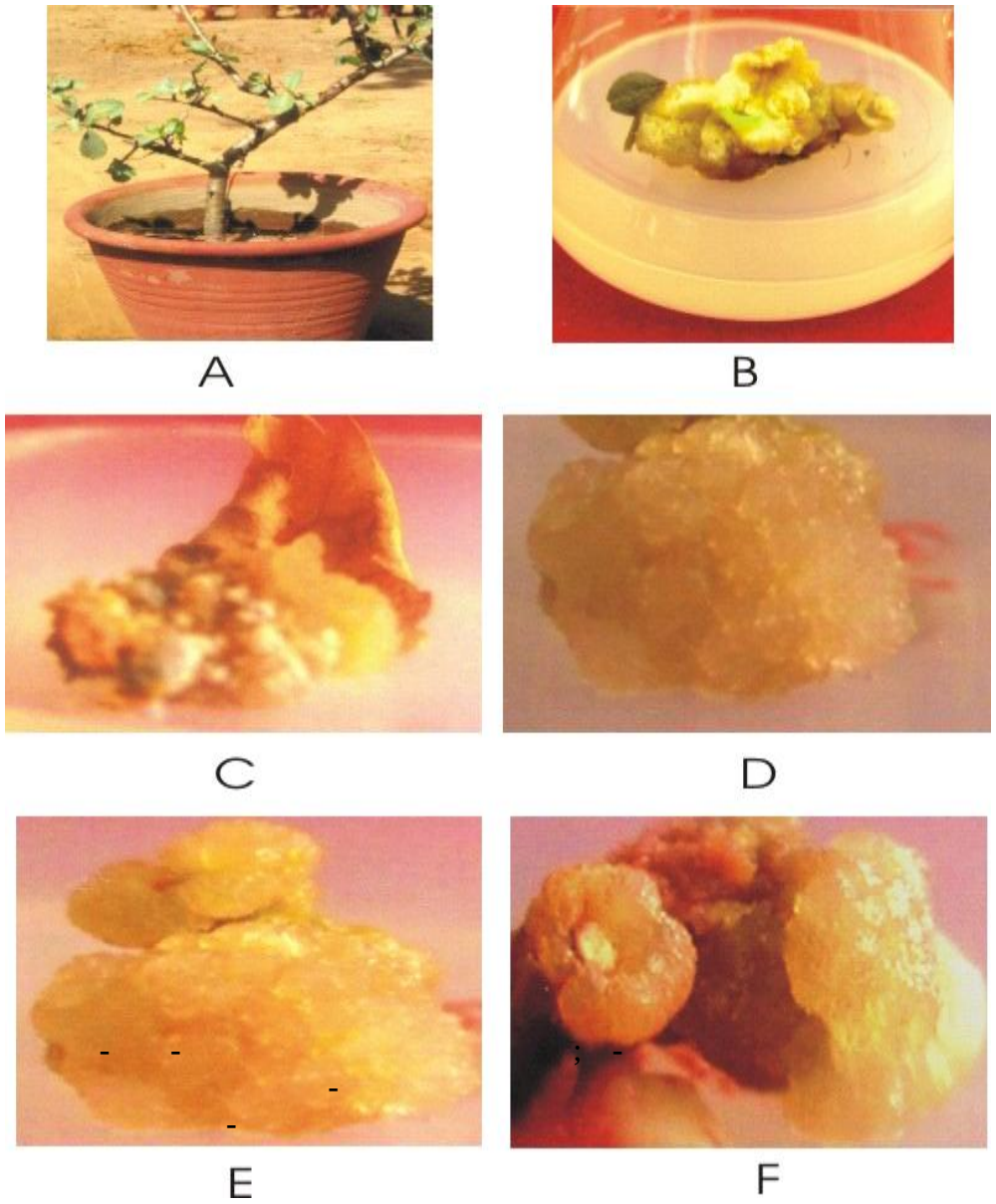

Figure: 1 A-F A-a matured plant of C. Wrightii; B- callus induced from embryo; C- callus induced from leaf; D- callus induced from nodal segment; E-12 weeks old callus from nodes; F-24 weeks old matured callus used for the extraction of guggulsterone.

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