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GROWTH OPTIMIZATION OF *COMMIPHORA WIGHTII* CALLUS -A POTENTIAL SOURCE OF *IN VITRO* GUGGALSTERONE PRODUCTION

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Commiphora wightii (Arn.) Bhandari also popularly known by the name *Guggul* is an endangered medicinal plant and chiefly known for its oleo-resin-gum (source of guggalsterones) which is being used medicinally since Vedic period and is a well-recognized drug in Indian Ayurvedic and Modern System of medicine. A study was carried out with a range of explants to see the effect of NAA and 2,4-D supplemented MS media on callus induction and biomass production. Guggalsterones have already been reported from callus tissues of *C. wightii*. Application of this study has the potential for selection of best explant and hormone concentration for high callus biomass production and it is evident that high biomass yielding calli may have higher guggalsterones content.

Keywords : Biomass; Callus; *Commiphora wightii; Guggul,* Guggalsterones.

Introduction

The genus *Commiphora* is widely distributed in tropical regions of Africa, Madagasker and Asia. The distribution further extends to Australia and the Indian Ocean and Pacific islands. In India it is widely distributed in Gujarat and Rajasthan[1]. The Guggul gum that is derived from the plant *C. wightii* is an important ingredient of a large number of Ayurvedic formulations. The National Chemical Laboratory, Pune, in collaboration with the CDRI, began a detailed chemical study of Guggul oleo-gum-resin and found out that it has a complex structure made up of various compounds such as lignans, lipids, diterpenes and steroids. Ten steroids have been isolated from the resin, among these guggalsterone-Z and guggalsterone-E have hypolipidemic effect. The demand of guggal gum is too high. In absence of sources for this gum within India pharmacies are importing oleo-gum-resin from Pakistan. During April 2002-March 2003 total value of guggal oleo-gum-resin imported from Pakistan is of Rs. Of the order of 12024421[4]. A big fraction of this much of Indian currency can be saved by producing active compounds guggalsterone-E and –Z *in vitro*. Mathur *et al.,* 2005[2] reported presence of Guggalsterones *in vivo* as well as *in vitro*. They prepared ethyl acetate extracts of leaf, stem, callus cultures and gum-resin and HPLC profile of these samples showed distinctly separated GugglateronesE and Z. We have also carried out UV spectophotometric analysis of callus derived from leaves explants and found the presence of Guggalsterones (unpublished data).

The present study was carried out with the aim to select explant and culture conditions for optimized callus induction and biomass yield, which can then be used for Guggalsterone production on commercial scale.

Material and Methods

A. Explant: Explants were collected from mature plant part as well as from *in vitro* raised seddling. Leaves and stem node were collected from mature trees growing at AFRI campus. Immature zygotic embryos were collected from Kaylana hills, Jodhpur. Mature seeds were germinated and hypocotyls and cotyledon explants were collected from one month old seedlings .

Leaves, stem node, mature seeds and immature zygotic embryos were first washed with running tap water, followed by washings with 2% phosphate free liquid detergent and rinsed with distilled water to remove all traces of detergent. These explants were than dipped in 2.5% solution of streptomycin. After that these are surface sterilized with 0.1% mercuric chloride solution (Hi Media). Leaves and mature seeds were exposed to sterilient for 3 min. and stem node and immature fruits were for 5-7 min. Now 4-5 proper washings were given to explants to remove all traces of sterilient. Afterwards immature fruits were excised with the help of secateurs and immature zygotic embryos were taken out and inoculated on media.

B. Culture medium and growth conditions: Murashige and Skoog's[3] media was used as a source of nutrient for different experiments. MS media was supplemented with

Table 1. Callusing response of mature explant after 20 and 40 days of inoculation.

Explant	Hormone [mg/l]	% callusing [SE]	Callus color & texture	Callus growth	Callus color & texture	Callus growth	Mean % biomass [SE]
			20days	20 days	40days	40days	40days
Leaf	0.5 2,4-D	33.3 [0.0]	creamish, watery	+	creamish brown	+	7.09 [0.21]
	1 2,4-D	44.4 [11.1]	creamish, watery	+	creamish brown	+	7.41 [0.27]
	2 2,4-D	33.3 [0.0]	creamish, watery	+	creamish brown	+	7.88 [0.49]
	0.5 NAA	33.3 [0.0]	creamish, watery	+	creamish brown	+	4.14 [0.27]
	1NAA	33.3 [0.0]	creamish, watery	+	creamish brown	++	4.51 [0.26]
	2 NAA	55.6 [11.1]	creamish, watery	++	creamish brown	+++	4.52 [0.31]
Stem node	0.5 2,4-D	nil	nil	nil	nil	nil	nil
	1 2,4-D	nil	nil	nil	nil	nil	nil
	2 2,4-D	11.11 [11.11]	creamish, shiny, crystalline	+	brown, tough	+	7.84 [0.34]
	0.5 NAA	44.44 [11.11]	creamish, watery	+	brown, tough	+	4.74 [0.18]
	1NAA	44.44 [11.11]	creamish, watery	+	brown, tough	+	6.30 [0.48]
	2 NAA	66.7 [0.0]	Creamish, watery	+	brown, tough	+	7.33 [0.35]

phytohormones, sucrose (3% w/v) and solidified with agar (0.8% w/v). The phytohormones used were α-Naphthalene acetic acid and 2, 4- Dichlorophenoxy acetic acid. The pH of the media was adjusted to 5.8 before autoclaving. Fourty milli liter of medium was dispensed per conical flask. Cotton plugs made up of non-absorbent cotton were used. Glassware, forceps, scalpels, media and distilled water were autoclaved for 20 minutes at 121°C and 15 psi pressure.

The cultures were incubated under 16 hours light (1400-lux light intensity using 40W florescent rods) and 8 hour dark period at a temperature of 26±2°C. The cultures were regularly sub-cultured every four weeks on fresh medium. Observations were recorded at weekly interval. All Experiments repeated twice.

C. Callus induction and maintenance: Explants were inoculated on MS media supplemented with different phytohormones. Callus was subcultured on same media after 20 days of callus induction for further multiplication. The non-embryogenic callus is compact, profuse and pale

Table 2. Callusing response of juvenile explant after 20 and 40 days of inoculation on different auxins.

Explant	Hormone [mg/l]	% callusing [SE]	Callus color & texture	Callus growth	Callus color & texture	Callus growth	Mean % biomass [SE]
			20days	20 days	40days	40days	40days
Immafure zygotic embroys	0.5 2,4-D	66.7 [0.0]	creamish, fragile	+	creamish brown tough	++	6.42 [0.46]
	1 2,4-D	77.8 [11.1]	creamish, fragile	++	creamish brown tough	++	7.89 [0.49]
	2 2,4-D	44.4 [11.1]	creamish, crystalline, shiny	++	brown, tough	++	6.57 [0.28]
	0.5 NAA	77.8 [11.1]	creamish, watery	+	creamish brown tough	++	5.59 [0.29]
	1NAA	77.8 [11.1]	creamish, watery	+	creamish brown tough	++	7.52 [0.39]
	2 NAA	88.9 [11.1]	creamish, watery	++	brown, tough	++	8.43 [0.48]

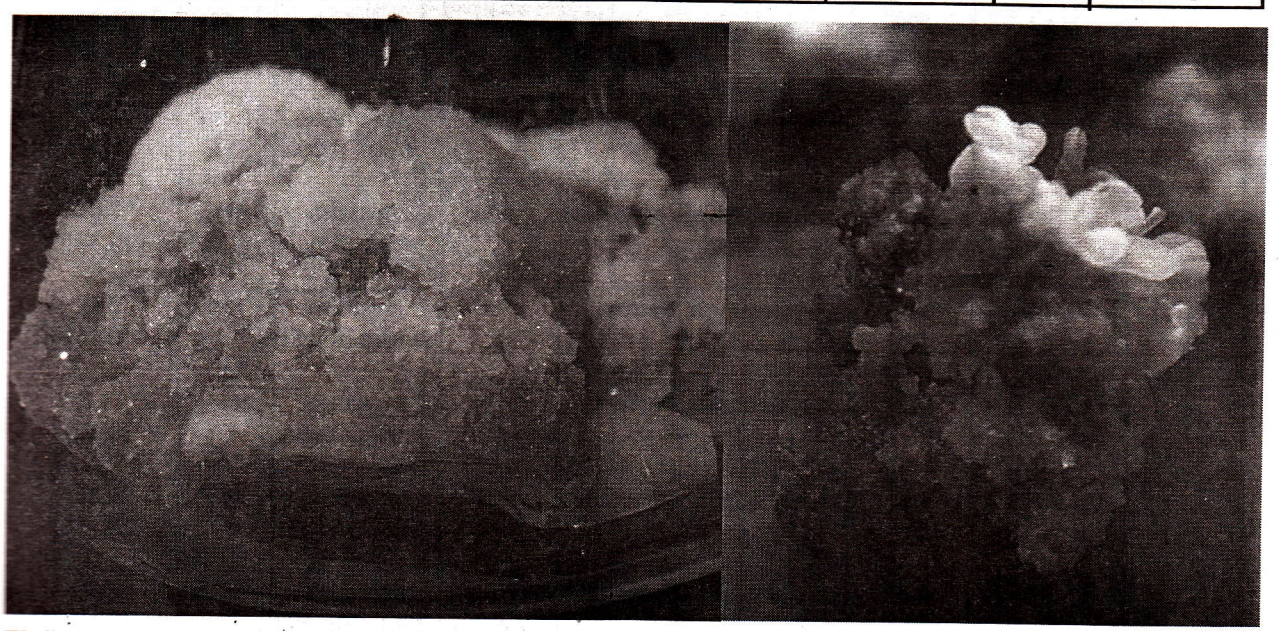

Fig.1. *Commiphora wightii* callus. A: Non-embryogenic callus; B: Embryogenic callus

in colour while embryogenic callus is fistinctly globular, greenish and compact (Fig. 1).

D. Biomass study: Callus sample was taken from cultures after 40 days of callus induction and fresh weight was recorded. Callus was dried at 65 °C for 48 hours in an electric oven and subsequently dry weight was recorded. Percentage biomass is calculated as follow:

% Biomass= (Dry weight/Fresh weight) X 100

Results and Discussion

Explants derived from mature plant (young leaves and stem nodes) showed better callusing response on MS medium supplemented with NAA as compare to 2,4-D (Table 1). With both the explants highest callusing was observed on 2 mg/l NAA. Same pattern was observed in explants derived from unriped fruits (immature zygotic embryos) (Table 2) as well as *in vitro* raised seedling

Table 3. Callusing response of *in vitro* raised juvenile explant after 20 and 40 days of inoculation on different auxins.

Explant	Hormone [mg/l]	% callusing [SE]	Callus color & texture	Callus growth	Callus color & texture	Callus growth	Mean % biomass [SE]
			20days	20 days	40days	40days	40days
	0.5 2,4-D	100 [0]	Creamish, ,watery	++	creamish-brown, fragile	+++	6.71 [0.63]
	1 2,4-D	100 [0]	Creamish, ,watery	++	creamish-brown, fragile	+++	6.88 [0.40]
	2 2,4-D	100 [0]	creamish,, watery	++	brown , tough	+++	7.75 [0.34]
	0.5 NAA	100 [0]	creamish, shiny, crystalline	++	creamish-brown, fragile	+++	6.1 [0.45]
Cotyledon	1NAA	100 [0]	creamish, shiny, crystalline	++	creamish-brown, fragile	+++	6.24 [0.22]
	2 NAA	100 [0]	creamish, shiny, crystalline	++	creamish-brown, fragile	Profuse	6.37 [0.37]
	0.5 2,4-D	88.9 [11.1]	creamish, shiny, crystalline	++	creamish-brown, fragile	+++	7.06 [0.34]
	1 2,4-D	66.7 [0]	Creamish,,watery	++	creamish-brown, fragile	+++	7.11 [0.26]
	2 2,4-D	55.6 [11.1]	creamish, watery	++	creamish-brown, fragile	+++	8.6 [0.30]
	0.5 NAA	100 [0]	creamish, shiny, crystalline	++	creamish-brown, fragile	+++	8.51 [0.33]
	1NAA	100 [0]	creamish, shiny, crystalline	++	creamish-brown, fragile	profuse	8.57 [0.17]
	2 NAA	100 [0]	creamish , shiny, soft	++	dark creamish, fragile	profuse	8.71 [0.24]

Table 4. Percentage biomass of *in vitro* raised juvenile explant after 40 days of inoculation on various hormone combinations (D = 2,4-D; N = NAA; B = BA; K = Kinetin).

Callus source	Auxin	Cytokinin		%Mean biomass [SE]
Hypocotyl 0.5mg/l 2,4-D	-	1B	-	5.12 [0.67]
	-	2B	-	5.19 [0.54]
	-	-	1K	4.54 [0.49]
	-	-	2K	6.75 [0.65]
	-	0.5B	1K	0.88 [0.23]
	-	1B	1K	8.19 [1.3]
	-	1B	1K	3.91 [0.68]
	-	2B	2K	4.85 [0.43]
	-	3B	2K	5.43 [0.66]
	-	2B	1K	7.89 [0.33]
	0.1D	0.5B	-	4.18 [0.9]
	0.1D	1B	-	4.15 [0.27]
	0.1D	2B	-	5.07 [0.49]
	0.1D	3B	-	5.33±0.8
	0.1N	-	0.5K	3.42 [1.4]
	0.1N	0.5B	-	4.2 [1.1]
	1N	-	5K	5.1 [2.0]
	1N	-	1K	10.7 [0.97]
	1N	5B	-	9.74 [0.73]
	1N	1B	-	8.87 [0.51]
Cotyledon 0.5mg/l 2,4-D	-	0.5B	1K	6.36 [1.2]
	-	1B	1K	11.8 [0.57]
	-	1B	2K	4.47 [0.55]
	-	2B	2K	5.38 [0.59]
	-	3B	2K	7.63 [0.33]
	0.1N	-	0.5K	3.7 [1.6]
	0.1N	0.5B	-	4.7 [0.81]
	1N	-	5K	6.1 [0.6]
	1N	-	1K	5.56 [1.8]
	1N	5B	-	7.34 [0.57]
	1N	1B	-	8.16 [0.17]
	-	3TDZ	-	4.34 [0.51]
	0.5N	3TDZ	-	6.36 [0.86]

explants i.e. hypocotyls and cotyledon. Seedling explants showed 100% callusing on all the three concentrations of NAA used (0.5, 1 and 2 mg/l).

Callus derived from all the explants grows actively for 20-25 days on all the hormone concentrations tried. After 20-25 days showed no growth in case of stem node explant (Table 1), moderate growth in leaf explant on 1 mg/l and 2mg/l NAA supplemented media. Immature seed explant showed poor growth after 20 days. Hypocotyls and cotyledon showed good growth on all the concentrations of both hormones (Table 3).

Maximum biomass (8.43%) accumulated in callus derived from immature zygotic embryos on 2 mg/l NAA. In case of mature explants callus derived from leaves on 2mg/l 2,4-D showed maximum biomass accumulation (7.8%). In case of *in vitro* raised explants cotyledon showed maximum (8.71%) biomass values on 2mg/l NAA supplemented MS medium.

The percentage biomass of the callus derived from immature zygotic embryos explant has been found to be greater than the rest of the explant derived callus that were screened in the present study. This may be possible due to higher biosynthetic activity of the callus. Mathur *et al.*[2] reported that guggalsterone-E is present in higher concentration than –Z in undifferentiated callus, obtained from immature zygotic embryos grown on MS medium supplemented with 2.26 µM 2,4-D and 0.46 µM Kinetin. Overall this study implies that callus produced by immature zygotic embryos yields higher biomass, therefore can be used for production of guggalsterones under culture conditions.

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