In vitro response, growth and maintenance of callus of *aquilaria agallocha* Roxb. (Thymelaeaceae)

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ABSTRACT

Aquilaria agallocha Roxb. (Thymelaeaceae) is a commercially important tree species of North East India. Efficient callus induction of the species was noted in Murashige and Skoog's (MS) medium supplemented with 4mg/l 2,4-D. Callus growth was better maintained in MS medium containing 2mg/l 2, 4-D + 0.5mg/l BAP. The maximum growth rate was recorded in the 10th weeks of culture on the same maintenance media. Total soluble proteins content of callus tissue during callus growth was (11.25±0.86 mg/gm fresh weight) recorded in the six weeks of subculture and then decline during subsequent passage of subculture. A positive correlation between callus growth total soluble proteins content of callus was observed. This perception of induction and maintenance of callus can be used for future study.

Key words: *Aquilaria agallocha,* Thymelaeaceae, callus, soluble protein content.

INTRODUCTION

Agar-attar tree *Aquilaria agallocha* Roxb. of family Thymelaeaceae is a superior quality agar wood (Gaharu) producing tropical rainforest tree species. This tree distributed particularly in Indonesia, Thailand, Cambodia, Laoes, Malaysia, Northern India, Philipines, Bangladesh, Bhutan, and Burma. In India it is mainly confined to the North-eastern hilly region and distributed in the hills of almost all the states of the region (Talukder, 2012). The plant is of great commercial importance due to its resinous heartwood which turns aromatic due to formation of highly valuable agar-attar as a result of infection by endophytic fungi (Shrivastava *et al*., 2008). Although, the process of oil formation is not yet fully understood, it is perceived to be formed by the reaction of the trees against fungal infection or injuries. Artificial wounding of tree trunk by nails or by cutting is common in Upper Assam, especially among the traditional growers, which may accelerate the oil forming processes (Saikia *et al*., 2012). Factors such as tree age, seasonal variations in growth, and environmental and genetic factors may also play an important role in oil formation (Ng *et al*., 1997). For extraction of agar oil from heartwood of the tree; the whole tree has to sacrifice. Owing to this, agar oil traders destroy many uninfected trees in search of agar wood. Due to this type exploitation, *A, agallocha* is now rarely found in wild habitat in North East India and considered as a critically endangered in India (IUCN 2009). Consequently, it is included in IUCN red data list of the year 2011 as vulnerable and at the edge of extinction from the natural forests (Saikia *et al.*, 2012). The oil also has medical value. It has been used as a stimulant, cardial tonic, carminatives, antiasmatics, aphrodisiac and stringent. It also acts as antibacterial and antifungal agents (Talukdar, 2012). Phytochemical studies revealed that leaf and bark of the plant contain alkaloid, polyphenol, saponin, flavonoid, glycoside, amino acids etc. (Dash *et al*., 2008). Considering these high commercial importance of the tree the present studies aimed to standardize an efficient, rapid and less expensive protocol for the production and maintenance of callus of essential genotypes.

MATERIALS AND METHODS:

Shoot tip, nodes and internodes taken from field grown 5 - 6 month old plants were used as explants. MS (1962) medium and different concentrations of growth regulators such as 2,4-D, BAP, NAA, IAA and coconut milk(CM) were used.

Table-1. Morphogenetic response of different explants of *A. agallocha* in MS medium supplemented with different concentration and combination of various growth regulators.

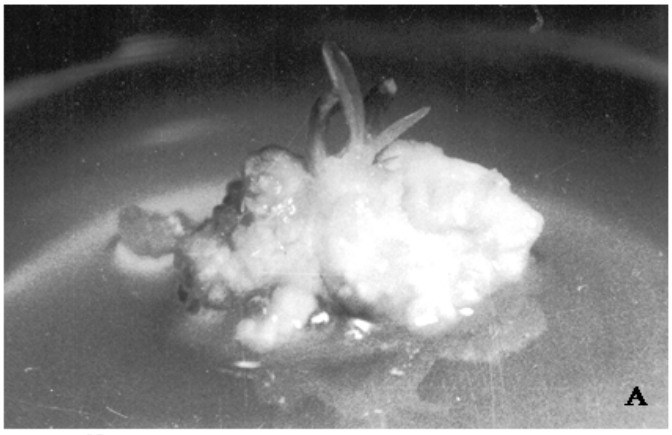
Growth regulators and adjuvant (mg/l)	Nature of response of explants after 21 days	
	Nodal segments	Young leaf
2,4-D(0.5)	Swelling	No response
2,4-D(1)	Callus, white, soft(+)	swelling
2,4-D(2)	Callus, white, soft(++)	Callus, green, soft(+)
2,4-D(4)	Callus, white, soft(+++)	Callus, green, soft(++)
2,4-D(1) + BAP(0.5)	Callus, creamy, soft(++)	Callus, green, soft(+)
2,4-D(1) + BAP(1)	Callus, creamy, soft(++)	Callus, green, soft(+)
2,4-D(2) + BAP(0.5)	Callus, creamy, soft(+++)	Callus, green, soft(+++)
2,4-D(1) + BAP(2)	Callus, green, compact(++)	Callus, green, compact(++)
2,4-D(1) + Kn(0.5)	Callus, green, soft(+)	Callus, green, soft(+)
2,4-D(1) + Kn(1)	Callus, green, soft(+)	Callus, green, hard(+)
2,4-D(1) + Kn(2)	Callus, green, compact(++)	Callus, green, hard(+)
2,4-D(2) + Kn(0.5)	Callus, green, soft(+++)	Callus, green, soft(++)
2,4-D(2) + CM(15% v/v)	Callus, white, soft(+++)	Callus, green, soft(++)
2,4-D(4) + CM(15% v/v)	Callus, white, soft(+++)	Callus, green, soft(+++)
NAA(1)	Swelling	Swelling
NAA(2)	Swelling	Swelling
NAA(4)	Swollen, hard, green	Swollen, hard, green
NAA(2)+BAP(0.5)	Callus, green, compact(+)	Callus, green, compact(+)
NAA(0.5)+BAP(2)	Swollen, hard, green	Swollen, hard, green
NAA(0.5)+BAP(4)	Initiation of shoot buds	Callus, hard, green(+)
NAA(0.5)+BAP(5)	Initiation of shoot buds	Callus, hard, green(+)
NAA(0.5)+Kn(4)	Callus, green, soft(+)	Callus, green, soft(+)
NAA(0.5)+Kn(5)	Callus, green, soft(++)	Callus, green, soft(+)
IAA(0.5)	Swelling	No response
IAA(2)	Callus, white, soft(+)	swelling
IAA(2)+BAP(0.5)	Callus, white, soft(++)	Callus, green, soft(+)
IAA(0.5)+BAP(2)	Callus, white, soft(+)	Callus, green, soft(+)
IAA(0.5)+BAP(4)	Callus, green, soft(++)	Callus, green, hard(+)
IAA(0.5)+Kn(4)	Callus, green, soft(++)	Callus, green, hard(+)
IAA(0.5)+CM(15% v/v)	Callus, white, soft(+)	Callus, white, soft(+)
BAP(0.5)	No response	No response
BAP(1)	No response	No response
BAP(2)	Swelling	Swelling
BAP(4)	Swelling	Swollen, hard, green
BAP(1)+CM(15% v/v)	Callus ,green, compact(+)	Callus ,green, compact(+)
BAP(2)+CM(15% v/v)	Callus ,green, compact(++)	Callus ,green, compact(+)
BAP(4)+CM(15% v/v)	Callus ,green, compact(++)	Callus ,green, compact(+)
BAP(2)+Ads.(0.5)	Swelling	Swollen, hard, green
BAP(4)+Ads.(0.5)	Initiation of shoot buds	Swollen, hard, green
BAP(5)+Ads.(0.5)	Initiation of shoot buds	Swollen, hard, green
Kn(4)+Ads.(0.5)	Swelling	Swollen, hard, green
Kn(4)	Swelling	Swelling
Kn(4)+CM(15% v/v)	Callus ,green, compact(++)	Callus ,green, compact(++)

Explants were washed thoroughly under running tap water for 10 to 15 min. Later plants were cut into pieces and washed with liquid detergent 5% Teepol (v/v) for 10 min and then sterilized with 0.1 % $HgCl_2$ solution for 5 min. followed by three to four rinses in autoclaved distilled water to remove traces of $HgCl_2$ under a laminar airflow. Small segments measuring 1 - 1.5 cm were cultured on MS medium supplemented with specific concentrations of growth regulators with 3% sugar. The media were gelled with 0.7 % agar with a pH of 5.8. Subcultures were done every 14 days interval. Cultures were kept for callus induction and maintained in various maintenance media. All cultures were kept at a temperature of 26±1^{0}C under 16 hour's photoperiod at 2000 - 3000 lux from fluorescent tubular lamps. To study the growth index the best callus derived from nodal explants were sub-cultured at an interval of 14 days. Growth index of the callus was measured by the formula: Final fresh weight minus initial fresh weight/initial fresh weight of the callus. The index was calculated from 14 – 112 days after first sub-culture of the callus tissue. Approximately 200 mgs callus tissue was taken as inoculums in each experimental set during study.

For estimation of total soluble protein during callus growth 100gm (fresh weight) callus tissue were taken from each subculture of callus grown in the different maintenance media at an interval of 21 days, up to a period of 105 days. Callus tissues were homogenized in 0.1M TRIS-HCl buffer (pH- 6.8) using a glass rod in mortar at an ice bath and centrifuge at 8000 rpm for 45 minutes at 4°C. The supernatant was collected and used for quantitative estimation of total soluble protein by the method of Lowery *et al.*, (1951) using BSA as a standard. The estimation is based on TCA precipitation count.

RESULTS AND DISCUSSION

Induction and growth of callus was noted in majority of experimental sets studied. Among the hormones treated 2,4-D (2mg/l to 4mg/l) alone as well as in combination with BAP(0.5mg/l) or CM(15% v/v) showed better initiation and growth of callus (Table-1).



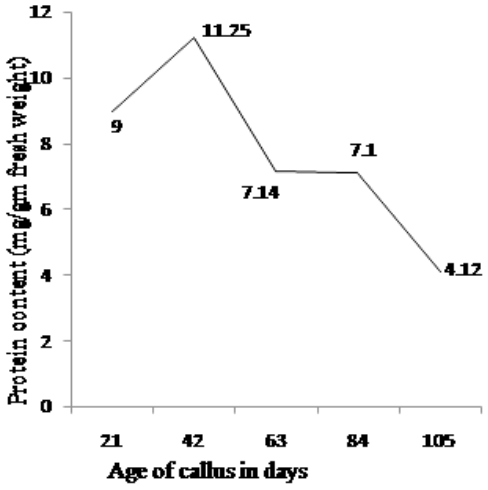


Chart-2

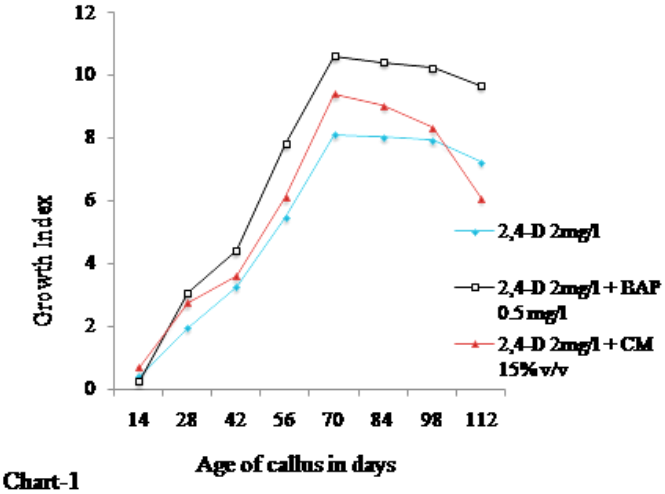


Chart-1

Fig-A. 42 days old callus on MS containing 2mg/l 2,4-D and 0.5mg/l BAP.
Chart-1. Comparison of growth indices of callus in different maintenance media.
Chart-2. Changes of total soluble protein content during callus growth in MS medium containing 2mg/l 2,4-D and 0.5mg/l BAP.

Table 2: Rate of callus growth in different maintenance media*

MS medium with growth regulators(mg/l)	Growth index in days							
	14	28	42	56	70	84	98	112
2,4-D(2)	0.42	1.95	3.25	5.46	8.10	8.02	7.90	7.22
2,4-D(2) + BAP(0.5)	0.95	3.05	4.40	7.82	10.65	10.42	10.25	9.65
2,4-D(2) + CM(15% v/v)	0.72	2.76	3.62	6.12	9.40	9.04	8.30	6.09

*Average of ten replicates

Table 3: Changes in the level of soluble protein content during callus growth (mg/gm fresh weight)

	Period in days				
	21	42	63	84	105
Protein content (Mean±SE)	9.0±14	11.25±0.86	7.14±0.12	7.10±0.20	4.12±0.06

Among the explants nodal segments were found most suitable for callus initiation in MS media supplemented with 4mg/l 2,4-D. Proliferation of the callus growth was better maintained in MS media containing 2mg/l 2,4-D and 0.5mg/l BAP (Table-2, Fig.-A). Maximum growth rate was recorded on same maintenance media containing in the 10^{th} weeks of culture (Chart-1). The texture and the colour of the calli were found to vary depending on the source of explants and the growth regulator supplemented in the media. The calli were found recalcitrant and there was no induction of shoot buds. Earlier worker (Talukder and Ahamed, 2001 and Saikia *et al*., 2012) observed maximum growth of callus in 45 to 60 days in lower concentration of auxin from leaf explants. Our result differs probably due to different explants source as well as use of higher concentration of auxin with lower concentration of cytokinine. The soluble protein content, which is generally considered as the important physiology parameter, was determined at different culture times of callus grown in best maintenance medium studied. Total soluble protein content (11.25 ± 0.86 mg/g fresh weight) during callus growth was the maximum in MS medium supplemented with 2mg/l 2,4-D and 0.5mg/l BAP in the sixth weeks of subculture and then decline slowly during subsequent passage of culture growth (Table-3, Chart-2). This may be due to the autolysis of cell contents and retardation of protein synthesis (Dey and Roy, 1987). In all the maintenance media used for recording the growth index of calli showed positive relationship between growth age and total soluble protein content of the calli. The present observation, therefore, demonstrate a possible means of callus initiation and maintenance from elite tree for future research (Lakshmi and Reddy, 2012 and Parveen *et al*., 2012).

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