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IN VITRO MULTIPLICATION OF SHOOT BUDS OF *AQUILARIA AGALLOCHA* ROXB. (THYMELAEACEAE)

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ABSTRACT

Direct induction of shoot buds from the nodal segments and shoot tips of agar-attar tree *Aquilaria agallocha* Roxb. (Thymelaeaceae) has been successfully achieved. Optimum number of shoot buds induction were observed from the nodal segments of juvenile tree in Murashige and Skoog's (MS) agar gelled medium supplemented with 4mg/l 6-Benzylaminopurine (BAP) and 0.5mg/l naphthalene acetic acid (NAA). Number of directly induced shoot buds increases up to 18 folds when it was sub-cultured in MS medium supplemented with 0.2mg/l BAP. Regeneration of roots was also achieved only when isolated shoots were initially soaked in higher concentration of IBA (40mg/l) in liquid half-MS for 48 hours and then sub-cultured in half-MS containing 2mg/l IBA for one week and finally transferred to auxin free medium in presence of 0.2% activated charcoal. The present study highlights the direct and rapid *in vitro* shoot multiplication of commercially important agar-attar tree.

Key Words: *Aquilaria Agallocha*, *Thymelaeaceae*, *In vitro* Regeneration, Shoot Buds.

INTRODUCTION

Agar-attar tree *Aquilaria agallocha* Roxb. Belonging to Thymelaeaceae (syn. *Aquilaria malaccensis* Lam) is a medium size tree, native to southwest Asia. Its distribution is recorded in rain forest of Indonesia, Thailand, Cambodia, Laos, Malaysia, Northern India, Philippines, 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 (Talukdar A. 2012). The tree is commonly known as agarwood, eagle wood or Gaharu. The term 'Agarwood' refers to its valuable resinous heart wood due to infection by few 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 caused by boring insects, or even manmade wounds. 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 collection of agar-attar or agar oil traders have to sacrifice whole tree as its heart wood serves as raw materials for oil distillation. Many uninfected or less infected trees are also been destroyed by them in search of agarwood. Due to such exploitation, this tree species is now rarely found in wild habitat in North East India and considered as critically endangered in India (IUCN, 2009). Consequently, it is included in IUCN red data list of the year 2011 as vulnerable and by now, is at the edge of extinction from the natural forests (Saikia *et al.*, 2012).

The plant is naturally propagated through seeds very easily but it has restricted period of seed viability and germinate immediately after maturity and their rate of germination is reduced with storage due to rapid decrease of moisture content of seeds during the first few hours of storage. Considering these high commercial importance of the tree, *in vitro* propagation of *Aquilaria* could be used as an alternative strategy to supplement the conventional methods of regeneration. The present studies aim at standardization of an efficient and less expensive protocol for *in vitro* multiplication of shoot buds.

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MATERIALS AND METHODS

Shoot tip and nodes taken from field grown 5 - 6 month old plants were used as explants. 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 different growth regulators and coconut milk with 3% sugar. The media were gelled with 0.8 % agar with 5.8 pH. Subcultures were done every 14 days interval. Cultures were kept for callus induction and maintained for shoot initiation, proliferation and elongation. Each proliferated and adventitious shoot was cut from the basal end and sub-cultured again for further multiple shoot induction. Regenerated multiple shoots were cut and individual shoots were placed in half strength MS containing different concentrations of IBA, NAA and IAA for root induction. All cultures were kept at a temperature of $26 \pm 1^\circ\text{C}$ under 16 hour's photoperiod at 2000 - 3000 lux from fluorescent tube lamps.

For somatic chromosome studies, root tips were pretreated in saturated solution of Para-dichlorobenzene at $10 - 12^\circ\text{C}$ for four hour followed by fixation in 1:3 acetic acid ethyl alcohol mixtures. The root tips were then kept in 45% acetic acid for 8-10 min. thoroughly washed with distilled water and hydrolyzed with 1N HCl at cold for 5 min. After washing with distilled water root tips were stained with 2% aceto-orcin for 2 hours and squashed in 45% acetic acid. Five metaphase plates from each plant were taken into account for karyotype analysis.

RESULTS AND DISCUSSION

In vitro propagation of the elite tree is quite difficult, because of the exudation of phenols by the excised edge of the explants into the culture medium. This results in browning of the medium, leading to cell necrosis due to phenol toxicity. In spite of this, nodal segments of *A. agallocha* were found most suitable for shoot bud induction and differentiation. Direct induction of shoot buds from the nodal segments and shoot tips were successfully achieved in cytokinin rich medium (Figure-A). Optimum number of shoot buds initiation per explants (5.0 ± 0.40) was noted in MS media supplemented with 4mg/l BAP and 0.5mg/l NAA (Table-1). After a period of 21 days 1 - 2 axillary buds developed while after 6th weeks of culture the number of shoot buds generated varied from 2 - 7 per explants. Length of the shoot buds varied from 0.5 - 1.0 cm. No further elongation was achieved in the same hormone supplemented media. Among the treatments (Table-1) higher concentration of BAP in combination with NAA supported better shoot formation and multiplication in nodal explants, though same concentration of Kinetin with BAP induce callusing. However, earlier report (He *et al.*, 2005) indicated lower concentration of BAP induces shoot buds formation. When directly regenerated shoot buds transferred on MS medium containing lower concentration of BAP (0.5mg/l) number of shoot buds increased up to 18 folds after a period of 42 days of culture (Figure-B). Further cycle of multiplication on the same media, regenerated shoot showed the poor growth. Single excised isolated shoot was found to elongate when they were cultured on half-MS in presence of 0.3% activated charcoal (Figure-C).

Rooting of regenerated shoots were achieved when isolated shoots were initially soaked in higher concentration of IBA (40mg/l) in liquid half-MS for 48 hours and then sub-cultured on agar gelled MS medium containing 2mg/l IBA for 10 days and finally transferred to auxin free half MS media in presence of 0.2% activated charcoal (Figure-D). Root produced through the *in vitro* regeneration were cytologically analyzed and somatic chromosome number was found to be $2n=16$ (Figure-E). The observation clearly indicates cytological stability at chromosomal level. The present observations, therefore demonstrate a possible means of plantlet regeneration and rapid multiplication of *A. agallocha*. This may help in studying clonal propagation from elite tree with desirable genotypes.

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Table 1: Relative response and rate of multiple shoot formation of different explants of *A. agallocha* after 6weeks

MS medium + growth regulator (mg/l)	% of shoot buds initiation	No. of shoot buds developed/explants (mean±SE)	
		Node	Shoot tips
BAP(4) + NAA(0.5)	75	5.0 ± 0.40	Growth and elongation of shoot
BAP(5) + NAA(0.5)	70	4.8 ± 0.38	Growth and elongation of shoot
BAP(4) + Ads. (0.5)	55	3.8 ± 0.40	4.0 ± 0.64
BAP(5) +Ads. (0.5)	55	3.7 ± 0.28	4.0 ± 0.46

*Mean of 20 replications

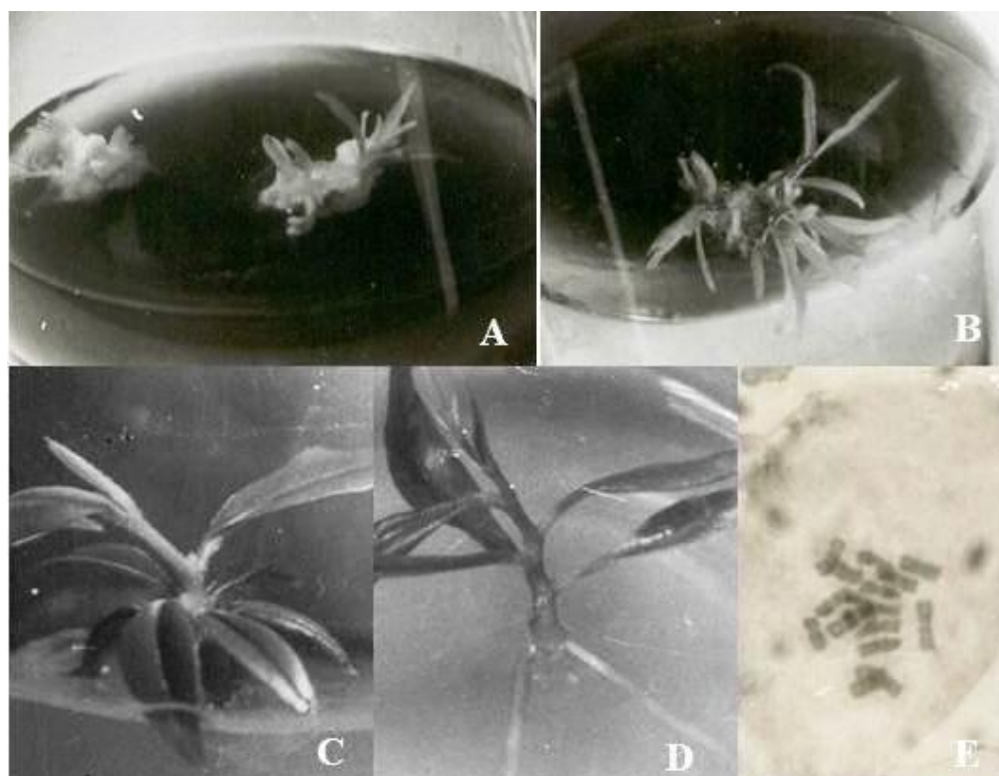


Figure A: Initiation of shoot buds from nodal segments in MS+BAP 5mg/l +NAA 0.5mg/l, B- Multiplication of shoot buds in MS+BAP 0.5mg/l, C- Elongation of shoot in hormone free MS medium, D- Rooting of regenerated shoot. E- Somatic chromosome number (2n=16) of regenerants.

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