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RESEARCH ARTICLE

IN-VITRO PROPAGATION OF *GARCINIA INDICA CHOISY* FROM SEEDLING EXPLANTS

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ABSTRACT

Garcinia indica, endemic to Western Ghats is a multipurpose tree of high priority for conservation. Garcinia (kokum) fruit is viewed as a Wonder berry and is known for its food, medicinal and commercial value. *In vitro* propagation, an ex situ conservation strategy, provides new means for conservation and mass propagation of economically important plants. The aim of the present study was to examine the main aspects of in vitro culture initiation and propagation of *Garcinia indica*, an endemic wild plant species. Successful initiation of tissue culture of garcinia was achieved from immature seeds. Germination was best achieved on half MS supplemented with 1.5mg/l GA_3. Murashige and Skoog (MS) medium supplemented with 6-benzyl amino purine (BAP) 3 mg/l along with Naphthalene acetic acid 0.5 mg/l produced shoots from the nodes within 4-5 weeks. Repeated and periodic sub culturing yielded better growth and further multiple shoots on the same medium. *In vitro* rooting was obtained on the half strength MS agar gelled medium supplemented with 2g/l activated charcoal 0.5mg/l NAA+1.5mg/l indole-3-butyric acid. The micro propagated plantlets were successfully acclimatized with survival rate 70%.

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INTRODUCTION

Garcinia indica, a plant in the mangosteen family (Clusiaceae), an endemic species to southern Western Ghats commonly known as *kokum*, is a fruit-bearing tree that has culinary, pharmaceutical, and industrial uses (Thatte *et al*., 2012; Utpala and nandakishore, 2014). It is also called Kokum butter tree, brindonia tallow tree or mangosteen oil tree. The tree grows up to 10-18 meters with drooping branches. It flowers from November to February with fruits ripening from April to May (Prle miland and Dhamija Isha, 2013). Fruits of *Garcinia indica* have been suggested in the Indian system of medicine for a number of diseases. These include its usefulness as an infusion, in skin rashes caused by allergies, to relieve sunstroke, remedy for dysentery, an appetizer, liver tonic, to allay thirst and as a cardio tonic. This plant is also pharmacologically studied for its anti-oxidative, chelating, free radical scavenging, anticancer, anti-inflammatory, and antiulcer activities (Sang *et al*., 2001; Chatterjee *et al*., 2003; Matsumoto *et al*., 2003; Hong J *et al*., 2006; 2007, Ramachandra, 2014). Conservation of plant biodiversity, a potential resource for health care, is essential for the very survival of the human race. Increasing importance to traditional medicine in the recent years has threatened the survival of rare species. Conventional plant propagation methods are affected by variable environmental factors and time consuming. In vitro propagation, an ex situ conservation strategy, provides new means for conservation and mass propagation of economically important plants. In addition, in vitro plant systems serve as an alternative approach for the production of bioactive compounds from medicinal plants (Vinoth *et al*., 2013). *Garcinia indica* species is listed as endangered species of southern India which makes it necessary to conserve this tree species (Swapna Dheodhar *et al*., 2014). Various workers have tried to regenerate garcinia plantlets using different explants such as apical bud (Deshpande *et al*., 1993, Mathew *et al*., 2001), root (Swapna Deodhar *et al*., 2008), Seeds (Malik *et al*., 2002) and leaf (Devendra Chauhan, 2012).

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MATERIALS AND METHODS

Plant source and seed germination

The fruits of *Garcinia indica* were collected from Western Ghat region India in the month of May, 2015. The collected fruits were first de pulped by using a sharp blade. Seeds were

first washed under running tap water for about 5 minutes and dipped in teepol (1:1 v/v) for 10 minutes. In order to minimize fungal contamination, seeds were treated with 1% (w/v) solution of Bavistin (Carbendazim 50% WP, a systemic fungicide) for 15 minutes, followed by proper rinsing with double distilled water for 3-4 times. Further sterilization was done under aseptic conditions in a Laminar Airflow Hood. Explants were surface sterilized with 0.1% (W/V) $HgCl_2$ for 10 minutes. Finally, the explants were washed thoroughly with autoclaved distilled water for several times to remove the traces of sterilant. Only healthy unwrinkled seeds were used for experimentation. Seeds were then inoculated aseptically on half MS medium containing 1.5mg/l GA_3, 3% sucrose and gelled with 0.6% agar-agar. From 3-6 weeks old seedlings, single node shoots segments were isolated and cultured on various combinations of phytohormones in MS medium.

Media preparation and culture conditions

MS (full strength) and MS/2 (half strength) basal salt media were prepared using sucrose (3%), agar-agar 0.6% and additives, viz. ascorbic acid (20 mg/l). Different combinations and concentrations of Growth regulators were incorporated into the media. The pH was adjusted to 5.7 & the culture bottles were capped with plastic cap and autoclaved at 121°C temperature for 15 minutes. For All the cultures were maintained at 25±2°C temperature and 70-80% relative humidity. The light intensity was maintained at 2000 lux using cool white fluorescent tubes with a16 h photoperiod.

Multiplication and Elongation

Nodal shoot segments as an explant were cultured on MS medium supplemented with cytokinins, viz. 6-benzyl amino purine (BAP) at different concentrations (1-5 mg/L) and Naphthalene acetic acid (0.5mg/L). The shoots initiated on the BAP were sub cultured for further shoot multiplication and elongation on MS medium supplemented with optimum concentration of BAP (3mg/L) along with NAA (0.5mg/L).

RESULTS

An Efficient protocol has been standardized for *In vitro* propagation of *Garcinia indica***.** The selected ex plants showed better germination compared to natural germination. The germinated shoots were then subcultured on MS medium supplemented with different concentrations and combinations of cytokinin and auxin (BAP and NAA; 1.0-5.0 mg/l). Medium containing BAP 3 mg/L and NAA 0.5mg/L was found to be the best concentrations for multiple shoot induction (65%) with the highest shoot number 8 and maximum shoot length was found to be 5 cm (Table 1).

Table 1. Effect of different concentrations of cytokinins (BAP) and Auxin (NAA) on multiple shoot initiation from nodal Shoot segments in MS medium after Six weeks of culture in *Garcinia Indica*

Sl.No	BAP mg/L	NAA mg/L	Response After 45 days	No. of shoots	Shoot length in Cm
1	1		60	3	5
2	2		65	5	5
3	3		65	8	5
4	4	0.5	65	8	4
5	5		60	6	3

Multiple shoots were developed within 45days (Figure B). *In vitro* rooting was obtained on the half strength MS agar gelled medium supplemented with 2g/l activated charcoal +0.5mg/l NAA+1.5mg/l indole-3-butyric acid. 70 per cent of survival of plants was observed during hardening (Figure F). All the plants transferred to polythene bags filled with sand + vermicompost + garden top soil (1:1:1, v/v) established well.

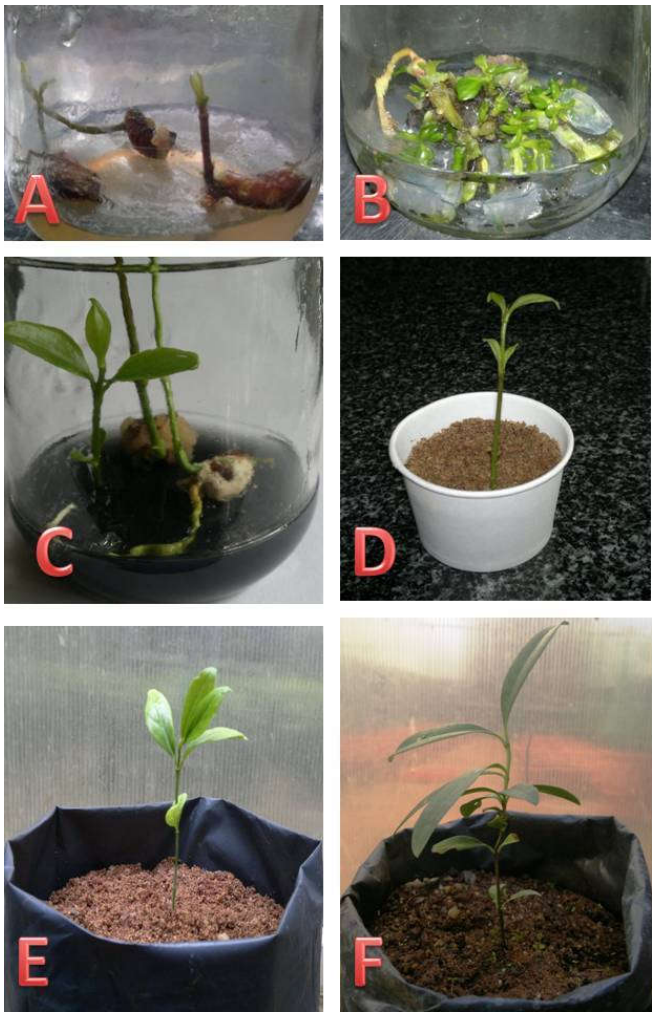

Figure 1. A- *In vitro* seed germination of *Garcinia Indica* in ½ Murashige and Skoog (MS) medium; B- Multiple shoot initiation from nodal shoot segments of *Garcinia Indica* in MS medium supplemented with benzyl amino purine (BAP, 3 mg/l)and NAA(0.5mg/l) C- *In vitro* rooting in MS ½ medium supplemented with 2g/l charcoal and indole-3-butyric acid (IBA, 1.5 mg/l); D- Primary hardening of *in vitro* raised plants; E- *Secondary hardening* after 30 days of hardening; F- *Secondary hardening* after 60 days of hardening

DISCUSSION

The result of present work correlates with the findings of various researchers. Normah *et al.* (1995) studied factors affecting *In vitro* shoot proliferation in *Garcinia* species. They selected seed as explants to produce plantlets. Kulkarni and deodhar (2002) also used immature seeds for *in vitro* production of *G.indica* plants. The present findings highlighted the different results in different combinations. The effect of plant growth regulators was significant on frequency of multiple shoot initiation, shoot number/explants and shoot length. With the increase in concentration of BAP, numbers of

shoots were increased, but shoot length was reduced at a higher concentration (4-5 mg/l). Thengane *et al.* (2006) reported 80% plant regeneration using immature seeds on medium Supplimented with different concentrations of cytokinin and auxin in combination. This present protocol is an attempt to conserve the endemic species of Garcinia by planting the invitro raised plantlets in medicinal garden maintained at M.M.Science and Arts College, Sirsi.

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