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Clonal Propagation of *Averrhoa carambola* Linn. Through Nodal Culture of Mature Tree

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Abstract: *Averrhoa carambola* Linn. was micropropagated from nodal explants through axillary branching on MS medium supplemented with benzyladenine (BA) and Kinetin. Maximum numbers of shoots per explant were obtained on MS medium supplemented with 1.0 mg l^{-1} each of BA and Kinetin. Subculturing of regenerated shoots in MS medium having low concentration of BA (0.1 mg l^{-1}) stimulates shoot elongation. Regenerated shoots were rooted by treating them with auxins and best root induction (70%) was observed in half-strength MS medium supplemented with 0.5 mg l^{-1} Indole-3-butyric acid (IBA). Rooted plantlets were successfully established in soil.

Key words: *Averrhoa carambola*, *in vitro* culture, clonal propagation

Introduction

Carambola (*Averrhoa carambola* Linn.) is an evergreen, woody fruit tree of humid tropics of the South and South-east Asia and is now being cultivated as a garden tree in all tropical and subtropical countries of the globe (Tidbury, 1976). There are commonly two formes of carambola, sweet and sour and both are grown in Indian subcontinent for variety of uses as ripe or unripe fruits (Singh, 1985). Ripe fruits are eaten in fresh, which are rich in reducing sugar, minerals, ascorbic acid and vitamin A and C (Ghose and Dhua, 1990). Carambola is mainly propagated through seeds and for being cross-pollinated, shows great variability in fruit quality; therefore vegetative propagation from selected clones is highly desirable. Clonal propagation of plants using tissue culture technique has many applications in agronomy (Murashige, 1974); especially for woody plants that are extreamly difficult to propagate by conventional means. Considerable reports describe the micropropagation of carambola using colyledon and seedling explants (Litz and Conover, 1980; Rao ***et al.***, 1982; Litz and Griffis, 1989; Khalekuzzaman ***et al.***, 1995a; Khalekuzzaman ***et al.***, 1995b; Islam ***et al.***, 1996). Here it is described the micropropagation of carambola using nodal buds from mature plants to provide an alternative way for its rapid clonal propagation.

Materials and Methods

Nodal segments were collected from physiologically mature shoots from higher crown of 16-20 years old phenotypically superior trees around the Rajshahi city, Bangladesh. After through wash with running tap water, the explants were immersed in 70% ethanol (1 min) and rinsed with

distilled water (2-3 time). The explants were finally surface disinfected with 0.1% (w/v) $HgCl_2$ (5 min) and rinsed in sterilized distilled water 4-5 times. Single node explants were then excised and cultured on MS medium (Murashige and Skoog, 1962) supplemented with different concentrations (0.5-2.0 mg l^{-1}) of cytokinin, benzyladenine (BA) or kinetin individually or in combination with others. The explants were subcultured in fresh medium in every two weeks and data were recorded after four weeks of second subculture. Elongated shoots (>3 cm) were excised and transferred to the rooting medium. The root induction media employed were half-strength and with various concentrations of auxins (0.2-1.0 mg l^{-1}; IBA, NAA & IAA). The pH of the medium was adjusted to 5.7 ±0.1 prior to autoclaving at 1.06 kg cm^{-1} (121°C) pressure for 20 min. Sucrose was used as carbon source at 40 g l^{-1} and all media were gelled with 0.7% agar (BDH). The cultures were kept under controlled conditions of temperature (25-27°C) and light provided by white florescent tubes during a 16h photoperiod. For each experiment a minimum 12-15 cultures were raised and each experiment was conducted two times.

Results and Discussion

Nodal segments were cultured on media supplemented with various concentrations and combinations of cytokinins showed different response during primary establishment. The response of explants to the treatments is presented in Table 1. Growth of nodal segments was noted within 12-21 days in culture. Callus formation was occasionally observed at the base of explants, but shoot proliferation occurred via axillary branching from buds present on the original explant (Fig. 1). Kinetin at 2.0 mg l^{-1} induced 42% shoot formation whereas it was enhanced to 60% at 2.0 mg l^{-1} BA. When BA and kinetin combination (1.0 mg l^{-1} each) was used most shoots were produced within five weeks and had the highest frequency of shoot formation (72%). 2ip was found to be least effective cytokinin for shoot formation. From the results obtained, MS medium supplemented with 1.0 mg BA and 1.0 mg l^{-1} kinetin was used as initiation and maintenance medium for shoot culture. Most of the nodal segments isolated from shoots produced in the initial cultures proliferated when subcultured on the fresh medium containing MS+1.0 mg l^{-1} IBA+1.0 mg l^{-1} kinetin (Fig. 2).

Table 1: Effect of various cytokinins on shoot proliferation from nodal segments of mature *A. carambola* tree

Cytokinins	Concentration mg l^{-1}	Days to bud sprout	Explants formed shoots (%)	No. of shoots per explant
Kinetin	0.5	14-20	22	1.8
	1.0	14-20	35	2.1
	2.0	16-21	42	2.6
BA	0.5	12-16	40	2.8
	1.0	12-16	49	3.4
	2.0	14-18	60	3.7
2ip	0.5	18-21	20	1.0
	1.0	18-21	26	1.5
	2.0	18-21	24	1.3
Kinetin+BA	0.5+0.5	12-14	64	4.3
	1.0+1.0	12-14	72	5.5

Table 2: Effect of auxins on root formation from *in vitro* grown shoots of *A. carambola*

Auxins	Concentration mg l^{-1}	Rooting (%)	No. of roots per shoot
NAA	0.2	-	-
	0.5	20	2.1
	1.0	25	2.6
IBA	0.2	40	3.6
	0.5	70	5.1
	1.0	65	4.7
IAA	0.2	-	-
	0.5	-	-
	1.0	20	2.3

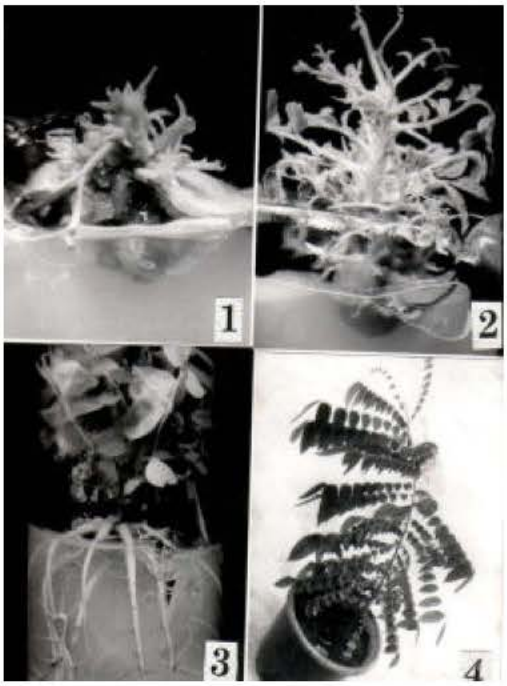

Fig. 1: Development of shoot buds from nodal segment on MS medium with 2.0 mg l^{-1} BA after two weeks of culture
2: Developments of multiple shoots on MS medium with 1.0 mg l^{-1} BA and 1.0 mg l^{-1} Kinetin, four weeks after culture
3: Initiation of adventitious roots from regenerated shoots on half-strength MS medium with 0.5 mg l^{-1} IBA
4: A plant established in pot after three months of transfer

To promote the elongation, shoots obtained from initial cultures were subcultured to MS medium supplemented with 0.1 mg l^{-1} BA, the shoots elongated and attained a height of 4-5 cm within 4-5 weeks. These elongated shoots were used for rooting experiment. Adventitious shoots

(>3 cm/long) were excised and cultured on half-strength MS medium containing various concentrations (0.2-1.0 mg l^{-1}) of auxins. Among the auxins used IBA found to be most suitable for root induction. Maximum (70%) roots were induced on half-strength MS medium with 0.5 mg l^{-1} IBA (Fig. 3, Table 2). NAA and IAA were less effective for root induction in *A carambola*.

Six-week-old plants from half-strength MS medium with 0.5 mg l^{-1} IBA were used in transplantation studies. About 60% plants survived following a method of pre-transplantation including a hardening stage. Micropropagated *A. carambola* plants, once established in soil, showed vigorous and uniform growth (Fig. 4). No morphological abnormalities have been observed.

The present study provides a method that insures a multiple shoot induction from nodal segments of mature tree of *A. carambola*. For multiple shoot formation required the presence of a cytokinin in the culture medium, which is in agreement with previous findings in other hardwood trees (Hutchinson and Zimmerman, 1987; Roy ***et al.***, 1993; Kabir ***et al.***, 1994; Islam ***et al.***, 1997 and Bansal ***et al.***, 2000. Effect of combined use of two cytokinins, BA and kinetin was superior in frequency and number of shoot formation to that of individual use of BA or kinetin. This synergistic effect of two cytokinins has been observed in Jackfruit (Roy ***et al.***, 1990) and in neem (Islam ***et al.***, 1997).

It was observed that a reduction in cytokinin concentration was favourable for shoot elongation. Ramesh and Padhya, 1990; Islam ***et al.***, 1997 observed similar effect during shoot proliferation in neem tree. The procedure described here appears to be adaptable for large clonal propagation of *A. carambola* and could be used in afforestation programmes in arid and semi-arid areas.

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