J. bio-sci. 15: 175-179, 2007 **ISSN 1023-8654**
http://www.banglajol.info/index.php/JBS/index **-Short Communication**

RAPID MULTIPLICATION OF *AVERRHOA CARAMBOLA* THROUGH *IN VITRO* CULTURE

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Averrhoa carambola L. (Family - Oxalidaceae) is a tropical common evergreen woody fruit plant. It has many commercially exploitable and beneficial attributes. The plant is a native to the south East Asia and presently being grown as a garden tree in all tropical and subtropical countries (Tidbury 1976). The fleshy sweet and sour fruits are widely eaten in Bangladesh. Carambola fruits are rich in reducing sugar, minerals and vitamin C and A (Ghose and Dhua 1990). Carambola is propagated by seeds. Cutting budding and grafting are unsuccessful. Characters of seed propagated plants vary widely among individuals. Micropropagation method is specifically applicable to species in which clonal propagation is needed (Gamborg and Phillips 1995). Rapid clonal propagation of desired genotype is one of many applications of plant tissue culture. Regeneration of plants *in vitro* from somatic tissue of *Averrhoa carambola* was first attempted by Litz and Conover (1980). This report was not satisfactory in multiplication rate and establishment of regenerated plantlets in soil. The objective of this paper is to establish an efficient and reproducible method for rapid clonal propagation of *Averrhora carambola* from aseptically grown seedlings.

Sweet and large type ripe fruits were collected from local bazaar for collection of seeds. After collection of seeds were brought to the laboratory. Then the seeds were washed thoroughly under running tap water to remove outer mucilaginous layer. Surface sterilization was done with 0.1 % $HgCl_2$ for five minutes and rinsed six minutes in sterile double distilled water. After these steps, seeds were cultured on MS basal medium for germination. Shoot tips and nodal segments were collected from 25 days old *in vitro* seedlings. Before implanting onto the culture media these were cut into small pieces (1.0 cm long each) for explants. MS (Murashige and Skoog 1962) basal media were used for regeneration of complete plant through *in vitro* culture. The basal medium consisted of the mineral salts and organic nutrient of the MS medium, 3% sucrose and 0.6% agar. Shoot tip and nodal segment explants were cultured on MS media supplemented with different concentrations and combinations of BAP alone or in combination with NAA or IAA for shoot regeneration. The regenerated shoots were rooted in half strength MS media supplemented with different concentrations and combinations of IBA alone or in combination with IAA. Effect of urea (50 -100 mg/l) on shoot development and effect of casein hydrolysate (CH) on shoot elongation were also determined. The pH of the media was adjusted to 5.8 before autoclaving. The cultures were maintained at 27?1?C under 16

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hours photo period. Five week old plantlets were taken out from the culture tube and washed to free from agar. Next, they were transplanted to small earthen pots containing sterile sand, soil and compost (1: 2 : 1) and covered with transparent polyethylene lid. The whole experiment was repeated three times and the cultures were regularly sub cultured on fresh medium at four week intervals.

Attempts to multiple shoot regeneration from shoot tip and nodal segment explants were cultured on MS media supplemented with different concentrations of cytokinin alone or in combinations of cytokinin and auxin. Shoot multiplication from nodal segment explant was observed when cultured in MS medium supplemented with BAP alone or, in combination with NAA or, IAA. The best medium leading to 85% explants producing 10 ? 0.2 shoot buds after four weeks consisted of MS medium containing 1.0 mg/l BAP and 0.5 mg/l IAA (Table 1, Fig 1). In the same medium, multiple shoot induction from shoot tip explant was low (data not shown). During the shoot proliferation stage, both axillary and adventitious shoots grew out from the explant and lateral branches developed on many of these newly formed shoots. In media containing only BAP or in combination with high concentrations of NAA, the explants produce low frequency of multiple shoots. Of the media supplemented with high concentrations of BAP and low concentrations of IAA, both explant types responded well and produced more shoots than the media containing only different concentrations of BAP (Table 1).

Table 1. Response of different concentrations of growth regulators supplemented in MS medium on shoot proliferation and multiplication from nodal explants of *Averrhoa carambola* (data were taken after four weeks of culture).

Growth regulators (mg/l)	Percentage of culture with induced shoots (%)	Number of shoots/culture
BAP 0.25	23 ? 1.5	3 ? 0.5
BAP 0.5	29 ? 2.0	3 ? 0.2
BAP 0.75	44 ? 0.5	4 ? 1.2
BAP 1.0	50 ? 1.5	6 ? 1.5
BAP 1.25	48 ? 0.8	5 ? 1.2
BAP 0.5 + NAA 0.25	55 ? 2.5	8 ? 0.2
BAP 1.0 + NAA 0.5	74 ? 1.5	9 ? 0.4
BAP 1.25 + NAA 1.0	63 ? 1.0	8 ? 1.5
BAP 0.5 + IAA 0.25	40 ? 2.5	5 ? 0.5
BAP 1.0 + IAA 0.5	85 ? 1.5	10 ? 0.2
BAP 1.25 + IAA 1.0	57 ? 0.5	7 ? 1.2

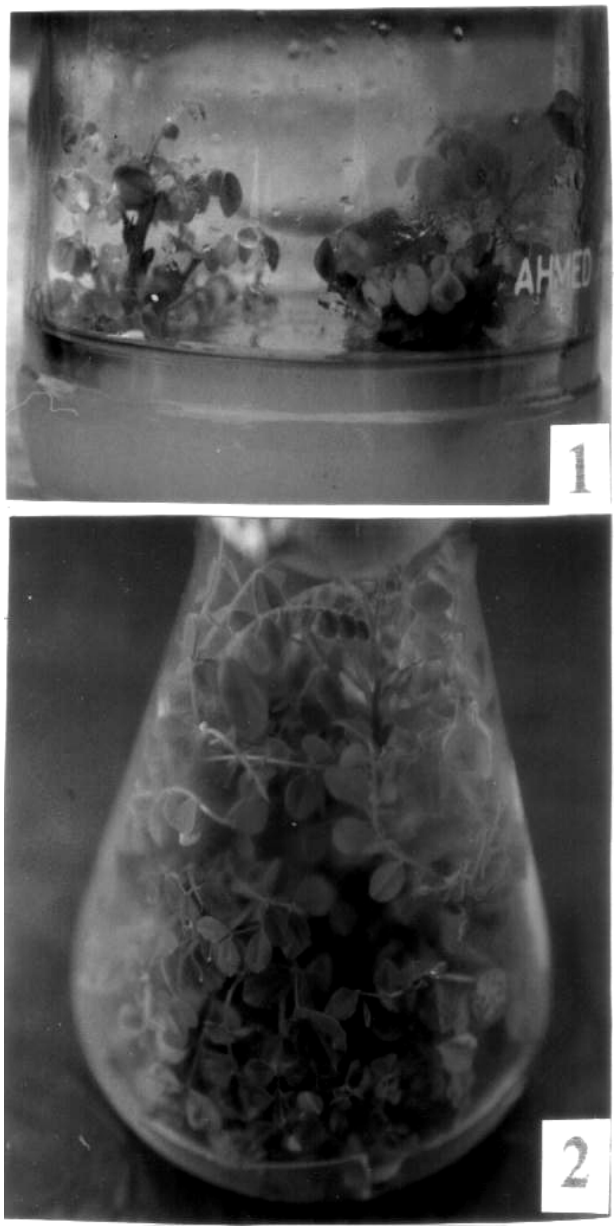

Fig. 1 Multiple shoot formation from the nodal explant of *Averrhoa carambola* on MS medium containing 1.0 mg/l BAP and 0.5 mg/l IAA.

Fig. 2. High frequency regeneration of shoots on MS medium with 1.0 mg/l BAP + 0.5 mg/l IAA + 75 mg/l urea.

Brand and Lineberger (1988) reported that adventitious shoot formed when shoot tip explants of mature *Liquidambar styraciflua* L. cultured on medium supplemented with 2.5 mg/l BAP + 0.1 mg/l NAA. Rao *et al*. (1989) reported that mulberry apical shoot explants proliferated and regenerated on MS medium with BAP (1.0 mg/l) and NAA (0.2 mg/l). In an attempt to enhance shoot proliferation, urea (50-100 mg/l) was added to the medium. Addition of 75 mg/l urea to the medium increased the number of shoots per culture. Thus the more effective medium determined for high frequency regeneration of shoots was MS + 1.0 mg/l BAP + 0.5 mg/l IAA + 75 mg/l urea (Fig 2). Shoot elongation was best achieved when the explants were cultured on the MS + 1.0 mg/l BAP + 0.5 mg/l IAA + 75 mg/l urea + 100 mg/l CH medium. Romberger *et al*. (1970) reported that urea was important medium factor in the culture of apical meristems and shoot tips of *Picea abies*. Addition of urea in culture medium was also fruitful in *Betula papyifera* culture (Minocha 1981). For root induction *in vitro* regenerated shoots were excised and placed into rooting media. Different concentrations and combinations of IBA, IAA and NAA were used in half strength MS medium for root induction. Best response was observed when 0.5 mg/l each of IBA and IAA were added in half strength MS medium (Table 2).

Table 2. Effect of IBA, IAA and NAA in half strength MS media on root formation from regenerated shoots of *A. carambola* (data were taken after 22 days of culture).

Growth regulators (mg/l)	Shoots rooted (%)
IBA 0.25	45 ? 0.5
IBA 0.5	75 ? 1.5
IBA 1.0	77 ? 0.2
IBA 1.5	55 ? 0.2
IBA 0.5 + IAA 0.25	60 ? 0.5
IBA 0.5 + IAA 0.5	88 ? 1.2
IBA 1.0 + IAA 0.5	70 ? 0.8
IBA 1.0 + IAA 1.0	62 ? 0.3
IBA 1.5 + IAA 1.0	53 ? 1.4

In this combination, it was observed that 88% shoots rooted within 22 days of culture. Roy *et al.* (1993) reported that IBA and NAA were essential for *Artocarpus heterophyllus* to root induction. Three auxins, IBA, IAA and IPA (Indole propionic acid) were fruitful combination for induction of root on *Tectona grandis* (Gupta *et al*. 1980). The well rooted plantlets were transplanted in small earthen pots. Individual plants were kept moist with regular watering in laboratory for 15 days after acclimatization, 85% of plantlets was successfully established in the open field. The result of the present experiment demonstrated a reproducible and efficient regeneration protocol through *in vitro* culture of *Averrhoa carambola*.

References

Brand M H and Lineberger R D (1988) *In vitro* adventitious shoot formation on mature phase leaves and petioles of *Liquidamber styraciflua* L. *Plant Science (Limerick)* **57:** 173-179.

Gamborg O L and Phillips G C (1995) Laboratory facilities operation and management. In: Gamborg O L and Phillips G C (eds.). *Fundamental Methods of Plant Cell, Tissue and Organ Culture.* Springer Berlin, NewYork, pp 3-20.

Ghose B and Dhua T S (1990) Carambola. In: Fruits: Tropical and Subtropical. Bose T K and Mitra S K (eds). Naya prakash, Calcutta, India, pp 785-794.

Gupta P K, Nadgir A L, Mascarenhas A F and Jaganathan V (1980) Tissue Culture of Forest trees : Clonal multiplication of *Tectona grandis* L. (Teak) by tissue culture. *Plant Sci. Lett.* **17:** 259-268.

Litz R E and Conover R A (1980) Partial organogenesis in tissue culture of *Averrhoa carambola*. *Hort. Sci*. **15:** 735.

Minocha S C (1981) Role of the source of nitrogen in the growth of shoot tips and callus cultures of woody plants in vitro. In: Proc. IUFRO. Sect. S2015. Int. Workshop 'In vitro' cultivation of forest tree species. *Fontainbleau France*: 227-235.

Murashige T and Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol. Plant* **15:** 473-497.

Rao P S, Bapat V A, Mhatre M and Patel G K (1989) Application of plant cell, tissue and organ culture in mulberry improvement programme. In: Sengupta K, Dandin S B (eds). Genetic resources of mulberry and utilization. Central Sericulture Research and Training Institue (CSR&TI), Mysore, pp. 125-130.

Romberger J A, Varnell R J and Tabor C A (1970) A culture of apical meristems and embryonic shoots of *Picea abies*. Tech. Bull. No. 1409, USDA For. Service: 30.

Roy S K, Islam M S, Sen J and Haddiuzzaman S (1993) Propagation of flood tolerant jack fruit (*Artocarpus heterophyllus*) by in vitro culture. *Acta Horticulturae* **336 :** 273-276.

Tidbury G E (1976) *Averrhoa* spp.- Carambola and Bilimbi. In: *The propagation of tropical fruit trees*. Garner R J and Chaudhary S A (eds). FAO, CAB, London. pp. 291-310.