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Micropropagation of *Artocarpus heterophyllus* Lamk

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A micropropagation protocol of Artocarpus heterophyllus *Lamk (Moraceae) was successfully established. Apical shoots from a 12-year- old* A. heterophyllus *tree were used as explants. The shoot explants that were cultured on Murashige and Skoog (MS) (1962) medium supplemented with 4.5 mg/L BA produced the most number of shoots (seven shoots per explant) after three to four weeks of culture. The* in vitro *shoots cultured on half strength MS medium supplemented with 0.5 mg/L NAA + 0.5 mg /L IBA and placed in total darkness for 10 days followed by light exposure for three weeks produced long, normal and healthy roots. About 70-80 per cent of the* in vitro A. heterophyllus *plantlets survived after acclimatisation and transferred to plant house.*

Keywords: *Acclimatisation, apical shoots,* Artocarpus heterophyllus *Lamk, micropropagation, multiple shoots, plantlets*

Jackfruit (*Artocarpus heterophyllus* Lamk.) is a tropical tree valued for its heavy yields of nutritious fruits and durable timber. It is one of the potential new crops, that has attracted increased interest in the world. Generally jackfruit is grown from seed but the seeds are difficult to germinate even just after a short period of storage (Singh, 1986; Samaddar, 1990). The conventional seed-propagated plants have high variation in plant progenies and rarely identical to the parent plants. Breeding the desirable genotypes using conventional vegetative propagation methods such as grafting and air layering are time consuming and difficult (Amin & Jaiswal, 1993). Besides, these techniques required skills, suitable plants source, good condition of rootstock and favourable environment. Tissue culture can hence be used as an alternative method for clonal propagation of selected *A. heterophyllus* genotypes to produce healthy plant materials for the growers in a more rapid and planned manner. In this study, we report a protocol for the micropropagation of *A. heterophyllus* plants.

MATERIALS AND METHODS

Establishment of aseptic explants

The apical shoots were taken from the actively growing branches of a 12-year-old *A. heterophyllus* tree grown in the Universiti Sains Malaysia campus, Penang, Malaysia. The apical shoots with intact cap-like stipule along with two to three axillary buds (about 3-5 cm length) were cut and placed in distilled water to maintain the freshness. The apical shoots were brushed using a soft brush with a mixture liquid detergent and 5.3 per cent sodium hypochlorite solution. They were then immersed in the same mixture solution with continuous agitation for one minute. This was followed by rinsing the explants three times with sterile distilled water. The apical shoots were then dipped into 95 per cent ethanol for one

minute to remove the latex secreted from the cut edges. They were then surface-sterilised in 20 per cent (v/v) of the 5.3 per cent sodium hypochlorite solution that was added with two drops of Teepol with continuous agitation for 10 minutes followed by rinsing three times with sterile distilled water. They were again surface-sterilised using 10 per cent (v/v) of the 5.3 per cent sodium hypochlorite solution added with two drops of Teepol for 10 minutes and rinsed three times with sterile distilled water. The stipules of the apical shoots were removed and the inner young shoots were used as explants.

Induction of multiple shoots

The cleansed shoot explants were cultured on Murashige and Skoog (1962) (MS) medium supplemented with benzyladenine (BA) at 3.0, 3.5, 4.0, 4.5 or 5.0 mg/L and Indole-3-butyric acid (IBA) at 0, 0.5, 1.0, 1.5 or 2.0 mg/L using a 5x5 factorial design. The Medium was prepared in 50 ml conical flasks with rubber stopper. The pH of the medium was adjusted to pH 5.7 – 5.8 with 1M sodium hydroxide (NaOH) or 1M hydrochloride acid (HCl), and added with 0.7 per cent agar (Algas, Chile) before autoclaved at 121°C and 1.05 kg/cm^2 for 13 minutes. Three aseptic shoot explants were inoculated vertically into each flask and six flasks were used for each treatment. The cultures were placed in a culture room regulated with a temperature of 25 ± 2°C under continuous light using cool white fluorescent tubes with light intensity of 30 μmol m^{-2}s^{-1}. The explants were sub-cultured into fresh medium every three weeks. Number of shoots survived, percentage of shoot explants produced multiple shoots, number of shoots formed from each explant and the shoot height were recorded after nine weeks of culture. The data were analysed using one-way analysis of variance (ANOVA) and comparison of means by Tukey Test (HSD) at p=0.05.

Rooting of *in vitro* shoots

Twenty *in vitro* shoots were inoculated into the rooting medium which consisted of half strength MS medium plus 1.5 per cent sucrose and supplemented with 1.0 mg/L NAA and 1.0 mg/L IBA. The cultures were placed in darkness (in a closed box) for 10 days. After 10 days, the shoots were sub-cultured onto a fresh rooting medium. These shoots were then exposed to light (30 μmol s^{-1} m^{-2}) for three weeks and the rooting response was then observed after four weeks. The experiment was repeated three times.

The rooted shoots were removed carefully (without breaking the delicate roots) from test-tube and rinsed with water to remove traces of the rooting medium. Each plantlet was trimmed down to two to three leaves, and planted in pot containing mixture of organic soil and top soil (1:1). All the pots were placed in a basin covered with transparent plastic sheet that was punched with little holes for aeration. The basin was placed inside the growth chamber regulated at 20 ± 1°C with 16 hours of photoperiod with light intensity of 30 μmol m^{-2} s^{-1}. After two weeks, the plastic cover was removed and the plantlets were kept in the growth chamber for another one week. Soil was kept at good drainage condition at all times. The plantlet were removed from the growth chamber and kept in the plant house. The survival rate and condition of the plantlets were recorded after one month in the plant house.

RESULTS AND DISCUSSION

The surface-sterilisation of the apical shoot explants of *A. heterophyllus*, using two stages

of 5.3 per cent sodium hypochlorite solution sterilisation technique, produced 100 per cent aseptic shoot explants. The cap-like stipule of the shoot explants protected the young inner shoot from contaminates and tissue damage from surface-sterilisation agents.

The percentage of the shoot explant formed multiple shoot on MS medium supplemented with BA (3.0 - 5.0 mg/L) was 66.7 - 100 per cent. With the presence of IBA (0.5 - 2.0 mg/L) in MS medium supplemented with BA (3.0 - 5.0 mg/L), less shoot explants forming multiple shoots when they were cultured in these media. All the shoot explants (100%) were found to produce multiple shoots when they were cultured on MS medium supplemented with 3.0 mg/L BA (*Figure 1*). This indicated that IBA was not needed for the induction of multiple shoots from the shoot explants of *A. heterophyllus*.

The number of shoots formed from each explant was high (4.2 - 7.0 shoot per explant) when the shoot explants were cultured on MS medium supplemented with BA only. With the presence of IBA, the number of shoots formed per explant was only three shoots or less. The multiplication of shoots decreased when the concentration of IBA in the MS medium increased from 0.5 to 2.0 mg/L with the presence of BA. The shoot explants cultured on MS medium supplemented with BA 4.5 mg/L formed the highest number of multiple shoots

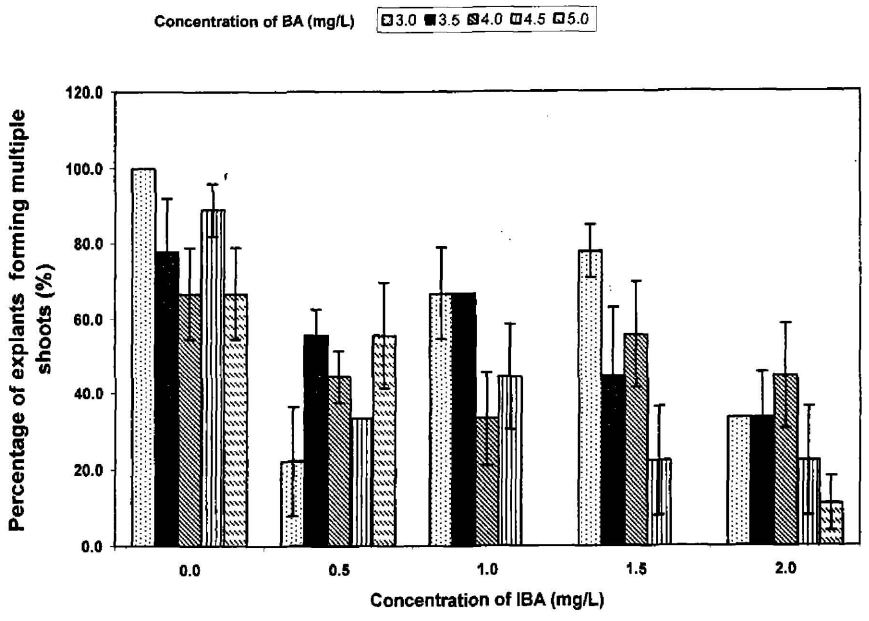

Figure 1 Effect of BA (3.0 – 5.0 mg/L) and IBA (0 – 2.0 mg/L) supplemented in MS medium on percentage of shoot explants forming multiple shoots.

(7.0±1.0 shoots per explant) within nine weeks (*Figure 2*). However, Amin and Jaiswal (1993) reported that MS medium supplement with BA and kinetin (1.0 – 2 mg/L) separately or in combination could induce six to ten new shoots from each explant within four to five weeks. While Roy *et al.* (1990) showed that MS medium supplemented with 1.0 mg/L BAP and 0.5 mg/L kinetin induced five to seven new shoots from each explant within four weeks. In our study, the concentration of BA added into the MS medium was about twice the amount used by Amin and Jaiswal (1993) and Roy *et al.* (1990). This could be due to the different cultivar and age of *A. heterophyllus* mother plant used in the experiment. In our study, the shoot explants were taken from a 12-year-old *A. heterophyllus* plant of Malaysian cultivar. The two researches used 30-50 years old *A. heterophyllus* tree of India cultivars. However, the number of multiple shoots formed from each explant was almost similar.

The shoots that were cultured on MS medium supplemented with BA (3.0 - 5.0 mg/L) and without IBA had higher survival rate (67 - 100%) compared to the shoot explants cultured on MS medium supplemented BA (3.0 - 5.0 mg/L) and IBA (0.5 - 2.0 mg/L). The percentages of shoot survival decreased as the concentration of both BA and IBA in the MS medium increased. For example, all the shoots survived when they were cultured on MS medium supplemented with 3.0 mg/L BA.

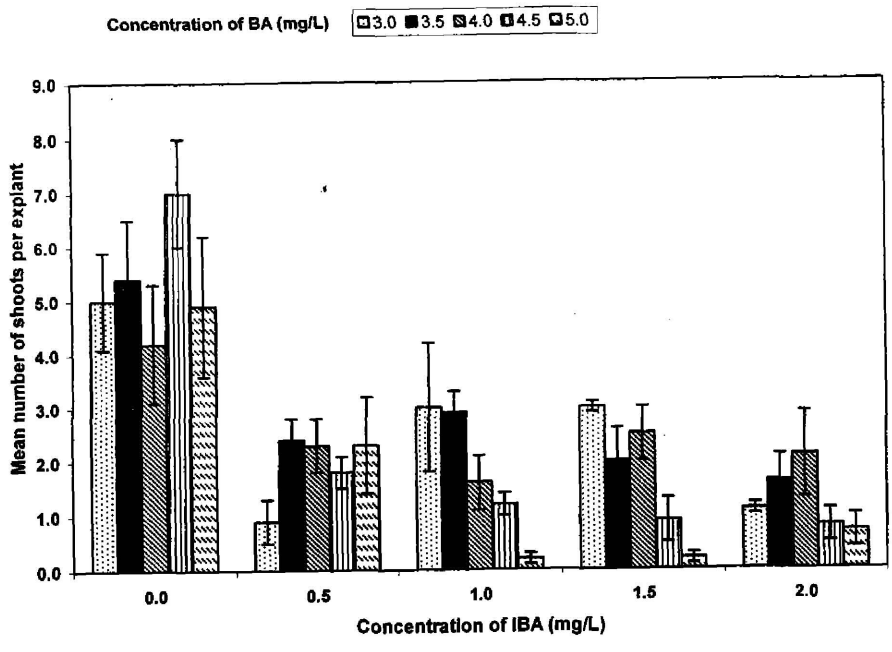

Figure 2 Effect of BA (3.0 – 5.0 mg/L) and IBA (0 – 2.0 mg/L) supplemented in MS medium on the formation of multiple shoots from each shoot explant

On the other hand only 22 per cent of the shoots survived when they were cultured on MS medium supplemented with 5.0 mg/L BA plus 1.0 – 1.5 mg/L IBA. The mean height of each induced multiple shoots ranged from 1 to 6 mm. Shoot height was slightly reduced with the presence of IBA (0.5 mg/L - 2.0 mg/L) in the culture medium (*Table 1*). The multiple shoots grew well with the formation of young shoots with succulent stem, long internodes, and light-green healthy leaves.

For successful rooting of the *in vitro* shoots, they had to be cultured on half-strength MS medium (1.5% sucrose) supplemented with 1.0 mg/L NAA and 1.0 mg/L IBA and incubated 10 days in total darkness. Only 42 per cent of the *in vitro* shoots produced roots after three weeks exposure to light. The new roots formed were healthy, but were short and thick (*Figure 3*). Lateral roots were formed at the fourth week. However, when the *in vitro* shoots were cultured on half-strength MS medium + 0.5 mg/L NAA + 0.5 mg/L IBA and sub-cultured to fresh medium after 10 days incubation in the

TABLE 1
EFFECT OF BA AND IBA IN MS MEDIUM ON THE SURVIVAL RATE AND SHOOT HEIGHT OF *A. HETEROPHYLLUS*

BA (mg/L)	*IBA (mg/L)*	*Percentage of shoot survival (%) ± s.e.*	*Mean shoot height (mm ± s.e.)*
3.0	0.0	100.0±0.0	4.6±0.6
	0.5	44.5±14.1	3.5±1.1
	1.0	77.8±14.1	2.7±0.1
	1.5	100.0±0.0	5.2±0.4
	2.0	77.8±7.0	4.0±0.4
3.5	0.0	88.9±7.0	4.8±0.5
	0.5	77.8±7.0	4.3±0.7
	1.0	77.8±7.0	4.8±0.2
	1.5	77.8±14.1	3.3±0.6
	2.0	55.6±18.6	2.8±0.9
4.0	0.0	66.7±12.2	5.9±0.8
	0.5	77.8±7.0	4.4±0.3
	1.0	55.6±18.6	2.4±0.9
	1.5	55.5±14.1	5.2±0.4
	2.0	44.5±14.1	2.6±0.8
4.5	0.0	88.9±7.0	3.9±0.2
	0.5	55.5±14.1	2.8±0.1
	1.0	66.7±0.0	4.1±0.3
	1.5	55.5±14.1	3.6±0.7
	2.0	44.5±14.1	2.7±1.0
5.0	0.0	77.8±14.1	4.1±0.2
	0.5	66.7±12.2	3.7±0.1
	1.0	22.2±14.1	1.2±0.7
	1.5	22.2±7.0	1.0±0.4
	2.0	33.3±12.2	1.2±0.5

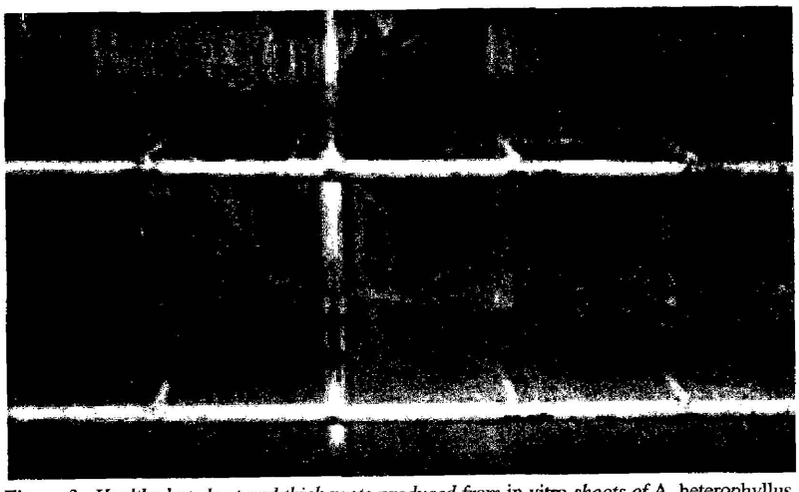

Figure 3 Healthy but short and thick roots produced from in vitro *shoots of* A. heterophyllus *cultured on half strength MS medium plus 1.5% sucrose supplemented with 1.0 mg/L NAA and 1.0 mg/L IBA after 10 days incubated in total darkness*

total darkness, 40 per cent of the shoots produced roots which were normal, long and healthy with abundant lateral roots after three weeks (*Figure 4*).

It was found that successful rooting occurred only when the *in vitro* shoots were incubated in total darkness for 10 days before subculture into the rooting medium and exposed to light for another three weeks. The *in vitro* shoots did not root if they were exposed to continuous illumination. This showed that lighting influenced the root initiation process. George and Sherrington (1984) suggested that there was an optimum concentration of internal carbon reserves for the formation of adventitious roots. The plant could obtain carbon source from the sucrose in the culture medium or by photosynthesis. If the carbon reserve was too high, rhizogenesis could be inhibited. When the *A. heterophyllus in vitro* shoots were placed in total darkness, the photosynthesis process was prevented. The

Figure 4 Normal and long healthy roots produced from in vitro *shoots of* A. heterophyllus *when cultured for 10 days in darkness on half strength MS medium supplemented with 0.5 mg/L NAA and 0.5 mg/L IBA*

sucrose in the culture medium was the only carbon source and was sufficient to cause root initiation with the aid of NAA and IBA (Auxin). When the shoots were cultured on MS medium with 3 per cent sucrose and supplemented with 1.0 mg/L NAA and IBA, no root was formed. However, *A. heterophyllus in vitro* shoots were able to root on MS medium plus 1.5 per cent sucrose with the same concentration of NAA and IBA. Therefore it could be assumed that sucrose at 3 per cent was too high for root initiation.

About 70-80 per cent of the rooted *A. heterophyllus* plantlets survived after being transferred to pots containing organic soil and top soil (1:1) mixture, which were placed in the growth chamber for three to four weeks.

CONCLUSION

Tissue cultured plantlets of nangka (*Artocarpus heterophyllus* Lamk.) could be produced from the apical shoots of a selected good quality mother plant using MS medium supplemented with 4.5 mg/L BA for multiple shoots induction followed by root formation on half strength MS supplemented with 0.5 mg/L NAA + 0.5 mg/L IBA. For normal roots formation, the rooting cultures should be placed in total darkness for 10 days followed by light exposure for three weeks before acclimatisation and transferred to plant house. The established protocol that took 12 weeks to produce the *in vitro* plantlets could be used for the commercial production of nangka clones

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