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EFFECT OF PLANT GROWTH SUBSTANCES ON THE *IN VITRO* PROPAGATION OF JACK (*ARTOCARPUS HETEROPHYLLUS* LAM.)

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Conventional methods of vegetative propagation (inarching, side grafting, air layering, budding and cutting) are possible for jack (Srinivasan, 1970; Dhua *et al.*, 1983); but the rate of multiplication is low and the methods cumbersome. *In vitro* methods are considered advantageous in this context.

An initial attempt for the micropropagation of jack was made by Rao *et al.* (1931). Rajmohan and Mohanakumaran (1983) presented a preliminary report on the *in vitro* propagation of jack, using explants from fresh stem-sprouts of five-year old trees. Detailed analyses were made of the effect of plant growth substances and other factors on the *in vitro* shoot proliferation via enhanced release of axillary buds and on the *in vitro* rooting of the explants from five-year old trees. The object was to develop a protocol for the successful micropropagation of jack, using explants from five-year old trees and to examine the applicability of the protocol to mature bearing trees.

Materials and Methods

Shoot spices from fresh stem-sprouts of five-year old jack trees were taken, dipped in 95 per cent ethyl alcohol for 10 s and thoroughly washed with sterile water. Surface disinfection was done by keeping the shoot apices in 2 per cent sodium hypochlorite solution for 30 min. Following a second washing with sterile water, the apices were agitated in a solution of 0.7 per cent insoluble PVP+2 per cent sucrose for 30 to 45 min and kept in sterile water at4-5°C for 24 h. They were again surface sterilised with 3 per cent sodium hypochlorite solution for 5 min and 0.1 per cent mercuric chloride solution for 10 min. A few drops of "Teepol" were added to the sterilants. The apices were rinsed (at least five times) with sterile water and trimmed to 1.5 cm length, aseptically. The explants were cultured on the establishment medium (MS medium supplemented with GA_3 1 mg/l and activated charcoal 1%) in darkness for four weeks with repeated subculturing. The cultures were exposed to light for four weeks, after which they were inoculated into the different media.

As the basal media for shoot proliferation, MS medium (Murashige and Skoog, 1962) and Anderson's medium (Anderson, 1980) were used. For *in vitro* rooting of the shoots, MS medium alone was used.

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A preliminary experiment with MS medium supplemented with 24 combinations of BA (2.5 to 40.0 mg/l) and NAA (0.1 to 0.8 mg/l) was conducted to standardise a basic proliferation medium (BPM) for the enhanced release of axillary buds from the shoot apices. In order to determine the effects of plant growth substances and other factors on the multiplication rate and growth of the shoot apex cultures, the BPM (minus the specific test factor) was supplemented with kinetin (2.5 to 40.0 mg/l), BA (2.5 to 40.0 mg/l), combinations of kinetin and BA (0.1 to 1.0 mg/l each), auxins like IAA, NAA and 2,4-D (0.1 to 0.4 mg/l each), cytokinin related substances like adenine and adenine sulphate (10 to 160 mg/l each), GA3 (1 to 16 mg/1), casein hydrolysate (50 to 2000 mg/l), carbon source like sucrose and glucose (1 to 4% each), inorganic salts of the MS medium (0.25 to 2 times the normal strength) or organic growth factors (inositol, pyridoxine, thiamine, glycine and nicotinic acid) of the MS medium (0.25 to 2 times the normal strength). The BPM with Anderson's basal medium was also tested. Another experiment with combinations of BA (1 to 4 mg/l) and IAA (0.1 to 0.4 mg/l) as well as BA (2 mg/l) and NAA (0.1 to 0.4 mg/l) was conducted to standaridse a medium which favoured elongation of the shoots in the cultures transferred from the proliferation medium. The shoots from the elongation medium were cultured for one to two weeks on MS medium supplemented with activated charcoal (0.1%) in reducad light intensity. From this culture, shoots (> 1 cm in length) were selected and used for the rooting experiment.

In vitro rooting of jack shoot cultures was attempted with MS medium (half concentration of mineral salts) supplemented with various auxins (IBA, NAA, IAA and 2,4-D) either alone or in combination. Direct transplanting of the shoots from the elongation medium was attempted by treating them with sterile IBA solutions (5 to 40 mg/l) for 24 h and then transplanting them to a standard potting mixture (sand : soil : cowdung 1:1:1 v/v). High humidity (90 to 100%) was maintained to favour establishment.

The pH of the media was adjusted to 5.7. Semi-solid media (30 ml. containing 0.6% agar in 100 ml conical flasks) were used for the studies. All the chemicals were of analytical grade. The cultures were incubated at 25 + 2°C with a 16 h photoperiod (1000lux) supplied by cool white fluorescent tubes. The cultures for the rooting experiment were incubated in the dark.

Results and Discussion

Culture establishment (Stage I)

The culture establishment medium containing GA_3 1 mg/l) and activatad charcoal (1%) recorded cent per cent survival and production of healthy, growing cultures of shoot apices, under dark conditions. The severity of the problem of polyphenol interference was minimised by incorporating activated charcoal (1%) and GA_3 (1 mg/l) in the establishment medium, insoluble PVP. in the proliferation medium and by frequent sub-culturing.

Shoot proliferation (Stage II)

The basic proliferation medium containing BA 5 mg/l and NAA 0.2 mg l induced maximum number of fairly elongated shoots (4.5 per explant). Higher concentrations of BA had a suppressive effect on the elongation of the axillary shoots (Table 1). The performance of kinetin and the combinations of kinetin and BA was not satisfactory, BA has been considered as the most effective cytokinin for stimulating axillary shoot proliferation in a number of species (Hu and Wang, 1983). Adenine and adenine sulphate, which are the precursors of endogenous cytokinins

Table 1

Effect of cytokinins on multiple shoot formation from jack shoot apices

Basal medium : MS + NAA 0.2 ppm

Treatment (concentration in ppm)				Shoots* per explant	Length of the longest* shoot (cm)	longest* leaf (cm)
BA	2.5			1.25	3.03	3.33
	5.0			4.75	2.15	2.90
	7.5			8.75	1.35	1.68
	10.0			7.75	1.08	1.28
	20.0			5.75	0.00	0.00
	40.0			0.00	0.00	0.00
Kinetin	2.5			1.25	2.23	2.53
	5.0			2.50	1.58	2.65
	7.5			3.50	1.18	2.50
	10.0			4.00	1.08	1.45
	20.0			3.00	0.90	0.60
	40.0			2.00	0.83	0.63
BA 0.1	+	Kinetin	0.1	1.00	1.05	1.78
0.1	+		0.5	1.25	0.98	1.83
0.1	+		1.0	1.00	1.40	2.33
BA 0.5	+	Kinetin	0.1	1.00	1.00	2.08
0.5	+		0.5	1.50	1.48	2.00
0.5	+		1.0	1.75	1.48	2.50
BA 1.0	+	Kinetin	0.1	1.50	1.50	2.18
1.0	+		0.5	1.50	1.43	2.60
1.0	+		1.0	1.50	1.43	2.65
		CD (5%)		1.62	0.40	0.21
		SEM +		0.57	0.14	0.07

Average of four observations Culture period: five weeks

Table 2

Effects of adenine, adenine sulphate, casein hydrolysate and GA_3 on multiple shoot formation from jack shoot apices

Basal medium : MS + BA 5 ppm + NAA 0.2 ppm

Treatment (concentration in ppm)		Shoots per explant	Length of the longest shoot (cm)	Length of the longest leaf (cm)
Adenine	10	5.50*	2.43*	3.08*
	20	6.00	2.50	2.93
	40	6.50	2.38	1.95
	80	8.00	1.85	2.05
	160	7.75	1.90	1.43
	0	5.50	2.15	2.90
	CD (5%)	1.56	0.30	0.45
	SEM+	0.52	0.10	0.15
Adenine sulphate	10	6.00*	2.65*	2.95*
	20	7.00	2.45	2.90
	40	8.75	1.95	1.98
	80	8.50	1.83	1.63
	160	8.00	1.35	1.73
	0	5.50	2.15	2.90
	CD (5%)	1.25	0.32	0.49
	SEM+	0.42	0.11	0.16
Casein hydrolysate	50	6.20**	1.92**	2.42**
	100	7.80	1.42	1.62
	500	8.40	1.16	1.26
	1000	8.00	1.00	0.86
	2000	7.40	1.00	0.58
	0	6.00	2.44	3.18
	CD (5%)	1.13	0.48	0.64
	SEM +	0.39	0.16	0.22
GA_3	1	5.20**	2.58**	3.56**
	2	5.60	1.92	2.82
	4	4.60	2.22	3.16
	8	5 00	2.30	2.40
	16	5.20	2.70	3.44
	0	5.20	2.44	3.16
	CD (5%)	NS	NS	0.63
	SEM +	0.37	0.28	0.22

* Average of four observations ** Average of five observations. Cultura period : five weeks

exhibited promotive effects on jack shoot proliferation. However, majority of the favourable levels of adenine and adenine sulphate affected the growth of the cultures by reducing the length of the shoot and the length of the longest leaf. Only adenine sulphate 20 mg/l recorded an increase in the multiplication rate (27.27%), without significantly affecting the growth of the cultures (Table 2). Increased rates of *in vitro* multiplication have been obtained earlier, by the action of adenine sulphate (Kitto and Young, 1981) and adenine (Nemeth, 1981).

The growth of the cultures, rather than the rate of multiplication, was improved by the auxins. In this respect, NAA 0.2 mg/l was superior to the levels of IAA and 2, 4-D, tried, nullifying the suppressive effects of the high cytokinin concentrations on the elongation of axillary shoots, the auxins might have restored normal growth of the shoots. Higher concentrations of auxins inhibited axillary and bud branching and induced callus formation. No beneficial effect, in term of the rate of shoot multiplication and growth of the cultures, was observed when GA, was included in the BPM (Table 2).

Casein hydrolysate (CH), a non-specific organic nitrogen source, has been known to increase the rate of *in vitro* proliferation of shoots (Mascarenhas *et al*. 1 Significant increase in the proliferation rate was observed in jack shoot cultures also (Table 2) However, the growth of the cultures was found to be severely affected. Similar inhibitory action of CH on shoot elongation was reported by Mascarenhas *et al*. (1981) in pomegranate.

Explants of certain fruit like red raspberry do not exhibit their maximum potential if grown on normal strength of MS medium, which is considered as a high salt medium (Anderson, 1980). However, in the case of jack the full strength MS medium was found to be the optimum (Table 3). Unlike in rhododendron (Anderson, 1980), the Anderson's medium, which is a low salt medium proved inefficient, in the present studies, to support the proliferation and growth of the jack shoot cultures.

Reducing the quantity of sucrose below the normal level (3%) was unfavourable in term of shoot multiplication rate and the growth of the cultures (Table 3). Increasing the quantity to 4 percent did not significantly improve the proliferation rate and the growth of the cultures. The requirement of sucrose (30-40 g/l) or glucose (20-30 g/l) may be related to the specific carbohydrate metabolism through which water relations and endogenous phytohormones are regulated.

In general, the conditions that stimulated shoot proliferation inhibited continued shoot growth. As the elongation of the majority of the jack shoots in Stage II was not satisfactory, an intermediate shoot elongation stage became necessary prior to Stage III, on MS medium containing NAA (0.2 mg/l) and reduced concentration of BA (2 mg/l) as in the case of *Sassafras* (Hu and Wang, 1983),

Table 3

Effect of MS inorganic salts, MS organic growth factors, sucrose and glucose on multiple shoot formation from jack shoot apices

Basal medium containing BA 5 ppm + NAA 0.2 ppm

Treatment		Shoots* per explant	Length of the longest* shoot (cm)	Length of the longest* leaf (cm)
Inorganic salts	0.25 conc.	2.20	1.08	1.12
	0.53 conc.	3.63	1.24	2.06
	1.00 conc.	5.20	2.42	3.06
	2.00 conc.	5.40	1.82	2.04
	CD(5%)	0.95	0.45	0.72
	SEM+	0.32	0.15	2.24
Organic growth factors	0.25 conc.	3.00	1.28	1.76
	0.5 conc.	3.00	2.08	2.12
	1.0 conc.	5.00	2.48	2.46
	2.0 conc.	5.40	2.20	2.26
	CD (5%)	0.90	0.69	NS
	SEM+	0.30	0.23	0.24
Sucrose	1%	3.60	0.92	1.60
	2%	4.60	1.26	2.76
	3%	5.40	2.24	2.92
	4%	6.00	1.80	2.70
	CD(5%)	0.79	0.62	0.59
	SEM+	0.27	0.21	0.20
Glucose	1%	3.40	1.52	2.28
	2%	5.00	2.14	3.06
	3%	4.60	2.00	2.70
	4%	3.40	1.58	2.54
	CD (5%)	1.04	0.50	0.51
	SEM+	0.35	0.17	0.17

Average of five observations Culture period : five weeks

Rooting of shoots (Stage III)

Maximum percentage of rooting (70%), maximum number of roots per shoot (5.43) minimum day for root initiation (13.43) and minimum callusing at the cut end of the shoots were recorded when the shoots were cultured for six days on half MS + IBA (2 mg/l) + NAA (2 mg/l), followed by transfer to half MS without the growth substances. Similar two-phase procedures helped in giving a strong root

induction stimulus to the hard-to-root apple rootstocks (Snir and Erez, 1980). The first phase is unfavourable for root elongation because of the high auxin content. The auxin-free second phase is ideal for root elongation. As observed in the present case, synergism between two auxins to yield better results in *in vitro* rooting has been reported earlier (Gupta *et al.*, 1980).

The concentration of inorganic salts in the basal medium influenced the *in vitro* rooting, regardless of the growth substances present. Half concentration of MS medium gave the maximum favourable effect on *in vitro* rooting of jack shoot cultures without affecting the shoot growth as in a number of species (Kartha *et al.*, 1981). Full strength of organic growth factors of MS medium was found to be the most favourable for root initiation. Sucrose was optimum at 3 g/l. As a source of energy and as a factor for osmoregulation for optimising the rooting response, sucrose has already been recognised (Chong and Pua. 1985). Agar 6 g/l was found to be the optimum for the *in vitro* rooting of jack shoot cultures. The concentration of agar is known to influence the rooting response in sensitive species (Kitto and Young, 1981). Attempts for direct planting out of the shoots taken from the proliferation medium (pre-treated with IBA solutions) were not successful.

Table 4

Effect of auxins on the *in vitro* rooting of jack shoot cultures

Basal medium : Half the concentration of MS inorganic salts and full concentration of MS organic growth factors

Treatment (concentration in ppm)	Root initiation	Roots per explant	Days for roots initiation
NAA 1.0	0	0	0
NAA 2.0	0	0	0
IBA 8.0	0	0	0
IAA 10.0	0	0	0
2, 4-D 0.1	0	0	0
NAA 0.05 + IBA 0.4	0	0	0
NAA 0.05 + IBA 0.2	0	0	0
NAA 0.4 + IBA 0.4	0	0	0
NAA 0.4 + IBA 0.8	0	0	0
NAA 0.4 IBA 1.6	80.0*	4.1	24.67
NAA 0.5 + IBA 0.2	0	0	0
NAA 2.0 + IBA 0.4	0	0	0
NAA 2.0 IBA 2.0 (for six days and then without plant growth substances)	70.0**	5.43	13.43
IAA 0.1 + IBA 0.1	0		

Average of 15 observations

Average of 10 observations

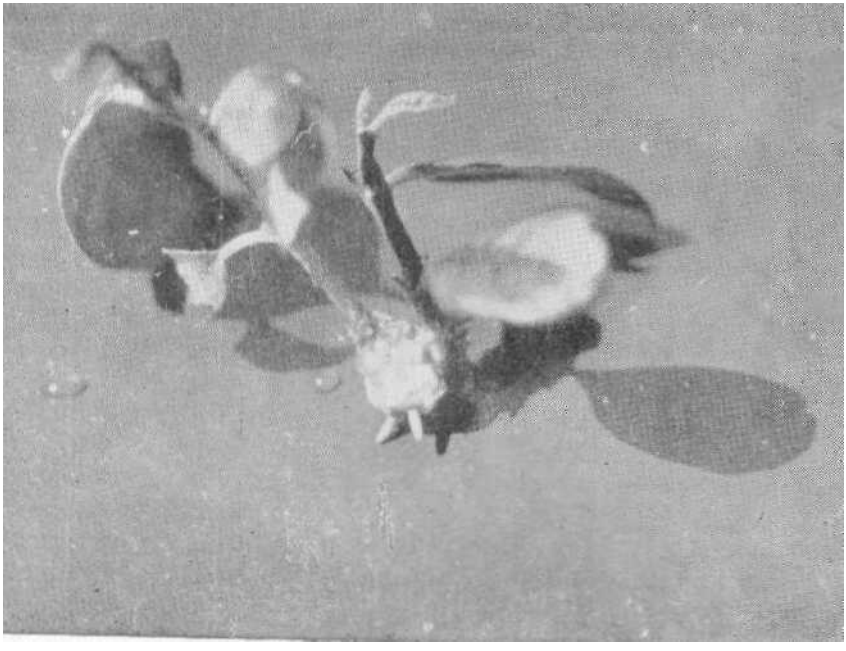

Plate I. Jack plantlets, just after root initiation

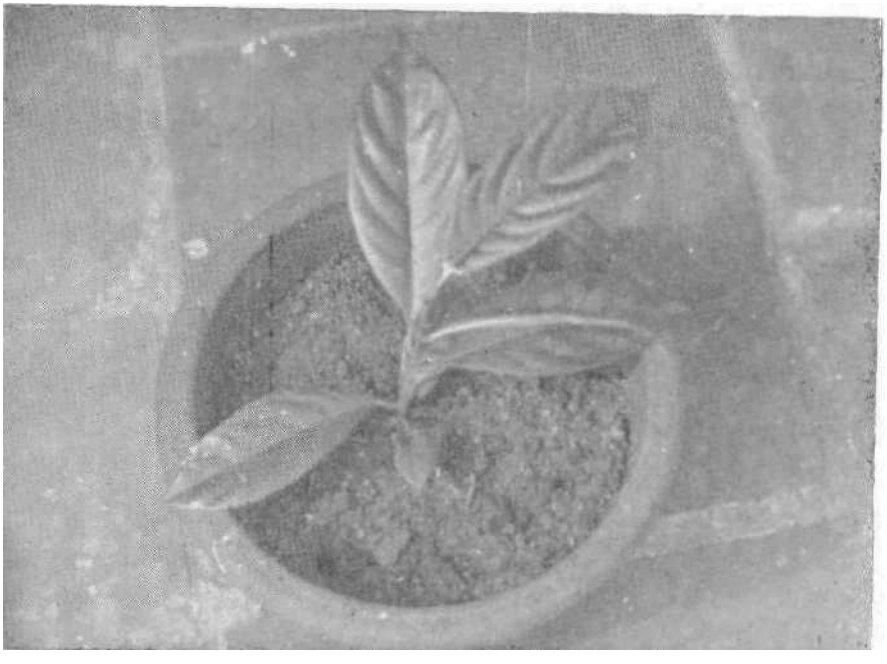

Plate II Jack Plantlet, three months after transfer to ordinary potting mixture

A normal chromosome count of 2n=56 was observed on cytological examination of the root tip squashes of the jack plantlets. Genetic stability has been observed in axillary shoots, due to the strict control over the mitotic events, by the meristematic line involved in their origin (Bonga, 1982).

Planting out (Stage IV)

After the appearance of the roots the plantlets were hardened by exposing them to high light intensity (3500 lux) for one week. The plantlets where then transferred to a vermiculite medium under high relative humidity (90—100%) and treated with a solution of the MS inorganic salts at half strength. A survival rate of 55.6 per cent was obtained after eight weeks. After another gradual hardening and as new leaves were produced, the plantlets were transferred to garden pots filled with a mixture of sand, soil and cowdung (1:1:1 v/v) and kept in the open field conditions.

Summary

Explants from shoot apices of fresh stem sprouts of five-year old jack (*Artocarpus heterophyllus* Lam.) trees registered a multiplication rate of 4.5 x. when cultured for five weeks on MS proliferation medium containing BA (5 mg/l) and NAA (0.2 mg/l). The normal strength of inorganic salts and organic growth factors of the MS medium, with 30—40 g/l of sucrose or 20—30 g/l of glucose was found to support the multiplication and growth of the cultures. GA_3 did not influence the shoot proliferation or growth. Adenine sulphate at 20 mg/l was found to increase the multiplication rate by 27.3 per cent, without affecting the growth. Adenine and casein hydrolysate were found not beneficial. Elongation of the shoots from the proliferation medium was observed on MS medium supplemented with BA 2 mg/l and NAA 0.2 mg/l. *In vitro* rooting of the shoots occurred when cultured for six days on a half strength MS medium containing IBA 2 mg/l, NAA 2 mg/l, sucrose 30 g/l and agar 6 g/l and then transferred to a similar medium without the plant growth substances. Cytological examination revealed the stability of chromosome number in the plantlets. When planted out, the plantlets registered 55.6 per cent survival.

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References

Anderson, W. C. 1980. Mass propagation by tissue culture : Principles and techniques. *Proc. Conf, Nursery Production of Fruit Plants through Tissue Culture: Applications and Feasibility.* U.S.D.A., Maryland, pp. 1—10.

Bonga, J. M. 1982. Vegetative propagation in relation to juvenility, maturity and rejuvenation. *In:* Bonga, J. M. and Durzan, D. J. (eds). *Tissue Culture in Forestry.* Martinus Nijihoff/Dr. W. Junk Publishers, London, pp. 150—181.

Chong, C. and Pua, E.C. 1985. Carbon nutrition of Ottawa 3 apple rootstock during stages of *in vitro* propagation. *J Hort. Sci.* 60: 285—290.

Dhua, R. S Sen, S K. and Bose, T. K. 1983. Propagation of jack fruit *(Artocarpus heterophyllus* Lam.) by stem cuttings, *Punjab Hort. J.* 23: 84—91.

Gupta, P, K . Nadgir, A. L., Mascarenhas, A. F. and Jagannathan, V. 1980. Tissue culture of forest trees: Clonal multiplication of *Tectona grandis* L. (teak) by tissue culture. *PI. Sci. Lett.* 17: 259—268.

Hu, C. Y. and Wang, P. J. 1983. Meristem, shoot tip and bud cultures. *In:* Evans, D. A., Sharp, W. R Ammirato, P. V. and Yamada, Y. (eds). *Hand Book of Plant Cell Culture.* Vol. I. *Techniques for Propagation and Breeding.* Macmillan Publishing Co., New York, pp. 177-227.

Kitto, S. L. and Young, M. J. 1981. *In vitro* propagation of Carrizo citfange. *Hort Sci.* 16: 305-306.

Kartha, K. K Mronginski, L. A Pahl, K. and Leung, N. L. 1981. Germplasm preservation of coffee *(Coffea arabica* L.) by the *in vitro* culture of shoot apical meristems. *PI. Sci. Lett.* 22: 301—357.

Mascarenhas, A. F. Gupta, P. K., Kulkarni, V. M., Mehta, U., Iyer, R. S., Khuspe, S. S. and Jagannathan, V. 1981. Propagation of trees by tissue culture. *In:* Rao, A. N. (ed). *Tissue Culture of Economically Important Plants.* COSTED, Singapore, pp. 175-179.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physio l. Plant.* 15: 473—497.

Nemeth, G. 1981. Adventitious root induction by substituted 2-chloro-3-phenyl-propionitriles in apple rootstocks cultured *in vitro. Scientia Hort.* 14: 253-259.

Rajmohan, K. and Mohanakumaran, N. 1983. *In vitro* rooting of jack shoot cultures. *Agric. Res. J. Kerala* 21(2) : 53—54.

Rao, N. K. S., Narayanaswamy, S., Chacko, E. K. and Doreswamy, R. 1981. Tissue culture of the jack tree. *Curr. Sci.* 50(7) : 310—312.

Snir, I. and Erez, A. 1980. *In vitro* propagation of Mailing Merton apple root-stocks. *Hort Sci.* 15: 597—598.

Srinivasan, K. 1970. "Muttom Varikka", a promising jack fruit variety. *Agric. Res. J. Kerala* 8(1) : 51—53.