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Factors affecting in vitro proliferation and rooting of shoots of jackfruit (*Artocarpus heterophyllus* Lam.)

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Abstract. Successful vegetative propagation of seedling jackfruit (*Artocarpus heterophyllus* Lam.) has been achieved by in vitro methods. Proliferation from nodal explants was greater than from shoot tips. Of the cytokinins tested, benzylaminopurine (BAP) was more effective than either 2-isopentenyladenine (2iP) or kinetin (Kin) and produced maximum proliferation when used at 5×10^{-6} M. Shoot proliferation was optimal at 30 °C with a 12 h photoperiod. Optimal rooting of shoots in vitro was obtained with indolebutyric acid (IBA) at 10^{-6} M. The number and length of roots was significantly increased in 12 h light as compared with the dark.

Introduction

The jackfruit is a popular fruit in some tropical and subtropical countries. The juicy pulp of the ripe fruit is eaten fresh or preserved in syrup. The pulp of the unripe fruit is used as a vegetable and the seeds are roasted or fried. The timber of the tree is considered superior to teak wood [3] and deserves greater consideration by foresters, especially as the fruit is a much-appreciated by-product [13]. It is mainly propagated by seed, which has resulted in immense variation in the population [11]. Conventional vegetative propagation methods are generally difficult, uneconomic and time consuming and mostly unsuccessful [8]. A successful method of propagation through tissue culture would be very useful for propagating selected varieties on a large scale in a short period of time. Therefore this study was carried out to determine optimum conditions for the proliferation and rooting of shoots by in vitro culture techniques. Early results of Rao et al. [10] indicated that some success had been achieved with induction of callus on hypocotyl segments with some subsequent regeneration.

Materials and methods

Seeds from a single jackfruit were germinated under sterile conditions and nodes from the shoots were cultivated and proliferated in a growth room at 29 ± 2 °C with 12 h light provided by Philips Colour 84 fluorescent lamps with a photon flux density of 85 μmol/m^2/s at the plant surface. These lamps provide more energy in the red end of the spectrum than warm white or daylight fluorescent lamps and may therefore allow more photosynthesis than normally occurs in cultured tissues.

The culture medium consisted of Murashige and Skoog [7] salts and vitamins at half the normal concentration. For shoot induction the medium was supplemented with 30 g/l sucrose and solidified with 6 g/l agar (oxoid purified). For root initiation the medium was supplemented with 20 g/l sucrose and solidified with 5 g/l agar. The medium was adjusted to pH 5.7 and sterilised at 121 °C and 103.5 kPa.

Experiment 1

Explants were used of shoot tip or first or second node from the apex cultured on shoot induction medium with BAP omitted or supplied at concentrations of 10^{-6}, 2.5×10^{-6}, 5×10^{-6}, 10^{-5}, 2.5×10^{-5}, 5×10^{-5} or 10^{-4} M. There were 10 replicates in each treatment. The number of shoots and the shoots above 1 cm were recorded after 6 weeks.

Experiment 2

Nodal explants were cultured in shoot induction medium without cytokinin or with BAP, 2iP and Kin supplied at concentrations of 5×10^{-6} or 5×10^{-5} M. There were 10 replicates in each treatment. Records of the number of shoots and of their fresh and dry weights were taken after 6 weeks.

Experiment 3

Nodal explants were grown on shoot induction medium with BAP at 5×10^{-6} M at temperatures of 20, 25, 30 or 35 °C with photoperiods of 0, 8, 12 and 16 h. There were 16 replicates in each treatment. Shoot number, shoots above 1 cm, and fresh and dry weights of shoots were taken after 6 weeks.

Experiment 4

The rooting efficiencies of indole-3-acetic acid (IAA), IBA, 1-napthalene acetic acid (NAA), 1-napthoxy acetic acid (NOA) and 2,4-dichlorophenoxy acetic acid (2,4-D) at 10^{-6} M were compared in 12 h light and in the dark.

A control treatment received no auxin. There were 10 replicate shoots, 4 cm ($\pm$ 0.5 cm) in length, in each treatment. The number and length of roots was recorded after 4 weeks.

Experiment 5
Similar to Experiment 4 with IBA omitted or supplied at 10^{-7}, 10^{-6} or 10^{-5} M in 12 h light and in the dark. Eight shoots were included in each treatment.

The experiments were of completely randomised or factorial design. The results were analysed by analysis of variance using Genstat statistical package produced by statisticians at Rothamsted Experimental Station for the University of London Amdahl computer. For discontinuous data, square root transformations were made and analysed, but the significances were little changed and therefore the analysis of the untransformed data was used.

Results

Experiment 1
Nodal explants gave more proliferation than shoot tips (Fig. 1a) but there was no significant difference between the two types of nodal explants. Although shoot tips proliferated less shoots, the proportion of shoots above 1 cm was increased. BAP at the lower levels significantly increased the proliferation of shoots but higher levels (5×10^{-5} and 10^{-4} M) inhibited shoot proliferation and development (Fig. 1b). The most effective concentration of BAP was 5×10^{-6} M.

Experiment 2
BAP was the most effective cytokinin in promoting shoots whilst 2iP and Kin were significantly less effective (Fig. 2a). The addition of cytokinin increased both fresh and dry weights over the control treatment, but the increase was significantly greater at the lower level of cytokinin (Fig. 2 b, c). BAP gave greater fresh and dry weights than either 2iP or Kin.

Experiment 3
Both the number and length of shoots above 1 cm were stimulated by increasing temperature up to 30 °C (Fig. 3a) although there was a slightly higher proportion of longer shoots at 25 °C than at 30 °C (Fig. 3b). The number of shoots was sharply decreased at 35 °C in all photoperiods, though

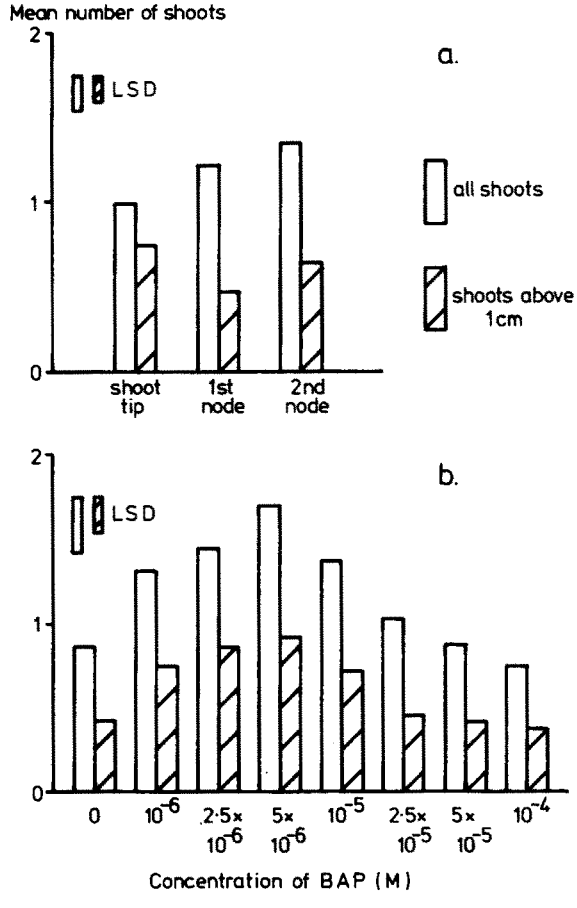

Fig. 1. The mean number of shoots per treatment produced after 6 weeks in culture. (a) Comparison of shoot tip explants with explants from first and second nodes. (b) Effect of a range of BAP concentrations summed over the 3 types of explant.

this effect was not shown in the dark (Fig. 3a). Different lengths of photoperiod had no significant effect on the proliferation and development of shoots, but there was less proliferation in the dark except at 35 °C. The weight of shoots was increased up to 30 °C both in the dark and at all photoperiods (Fig. 3c, d). The maximum fresh and dry weight occurred in the 12 h photoperiod at 30 °C. Although the fresh and dry weights continued to increase in the dark when the temperature was raised from 30 to 35 °C, there was a sharp decrease shown in all the light treatments. High temperature accompanied by light was more inhibitory than high temperature and dark, which was confirmed by using temperatures up to 40 °C [9].

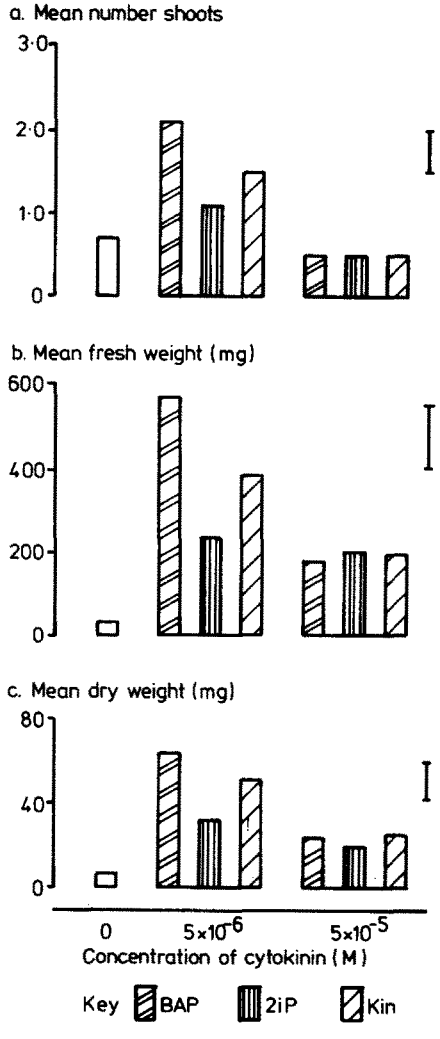

Fig. 2. The effects of cytokinins, BAP, 2iP, Kin, on the proliferation of shoots from nodal explants after 6 weeks (mean of 10 replicates per treatment). (a) Number of shoots per explant. (b) Fresh weight per explant (mg). (c) Dry weight per explant (mg). (Vertical range bars indicate LSD at 5%).

Experiment 4

Both IBA and IAA produced significantly more roots than when auxin was omitted or when NAA, 2,4-D or NOA were present (Table 1a). 2,4-D was very inhibitory to rooting and the cultures produced much soft white callus. The length of roots was also significantly greater with IBA than with other

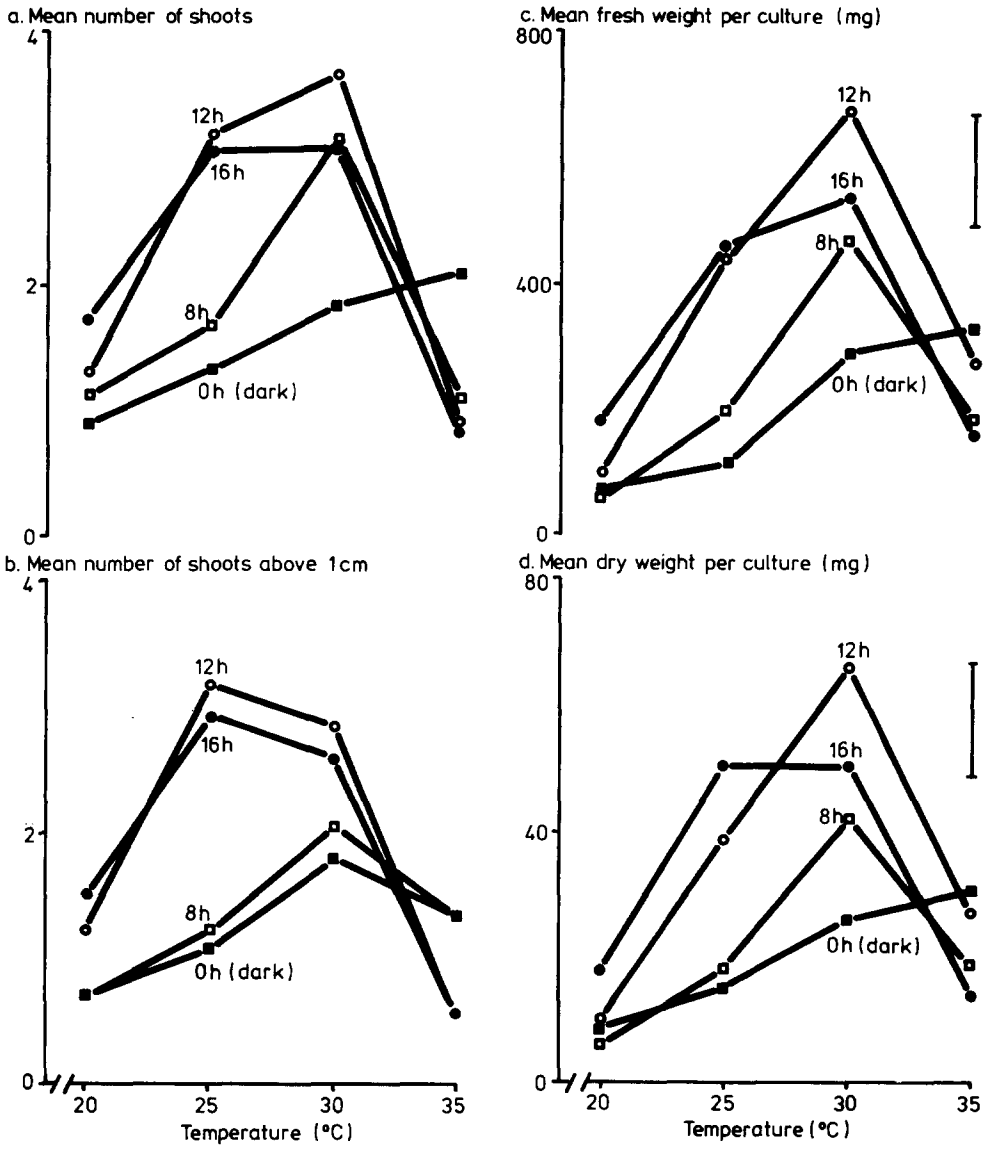

Fig. 3. The effects of temperature and photoperiod on nodal explants grown on shoot induction medium with BAP at 5×10^{-6} M. (Mean of 16 replicates per treatment). (a) Number of shoots per explant. (b) Number of shoots above 1 cm per explant. (c) Fresh weight per explant (mg). (d) Dry weight per explant (mg). (Vertical range bars indicate LSD at 5%).

auxins (Table 1b), although IAA produced a large number of very short roots. Both the number and length of roots were significantly increased when cultures were incubated in 12 h light as compared with the dark.

Table 1. Effect of various auxins on the initiation and growth of roots on cultured shootlets after 28 days (mean of 10 replicates per treatment).

a. Number of roots				*b. Length of roots (mm)*		
Type of auxin	Photoperiod		Main effect of auxin	Photoperiod		Main effect of auxin
	dark	12 h		dark	12 h	
None						
2.1	3.4	2.7	27	47	37	
IBA	4.5	6.3	5.4	28	64	46
NAA	1.7	3.6	2.6	18	43	30
IAA	5.2	6.4	5.8	17	36	27
2,4-D	0.3	0.2	0.2	0	0	0
NOA	2.5	1.8	2.1	16	17	17
Main effect of photoperiod	2.7	3.6		18	35	

Factors	Level of significance	LSD (0.05)	Level of significance	LSD (0.05)
Type of auxin	***	1.5	***	15
Photoperiod	*	0.8	***	08
Interaction			n.s.	

n.s. Not significant; $*p < 0.05$; $***p < 0.001$.

Experiment 5

The mean number of roots produced increased, with increasing concentration of IBA, significantly over the control without IBA (Table 2a). This effect was shown more clearly in the light than in the dark, though the interaction was not statistically significant. The length of roots per plantlet was increased significantly by the addition of IBA up to 10^{-6} M, but the highest level (10^{-5} M) was significantly inhibitory to root extension (Table 2b). Both the number and length of roots were significantly increased in 12 h light as compared with the dark.

Discussion

Although shoot tips proliferated less than nodal explants, the proportion of shoots exceeding 1 cm was increased. These effects may be attributable to variation in the endogenous auxin level of buds in different regions of the stem [5]. BAP was found to be more effective than 2iP or Kin on the proliferation and development of jackfruit shoots, an effect similar to that observed by Lundergan and Janick [6] in apple. Wareing and Phillips [14] showed that synthetic cytokinins, such as BAP, were more active than naturally occurring cytokinins, such as 2iP, in shoot proliferation. A BAP

Table 2. Effect of different concentrations of IBA on the formation and development of roots on cultured shootlets after 28 days (mean of 8 replicates per treatment).

a. Number of roots				*b. Length of roots (cm)*		
Concentration of IBA (M)	Photoperiod		Main effect of IBA concentration	Photoperiod		Main effect of IBA concentration
	dark	12 h		dark	12 h	
0	0.6	1.9	1.2	07	47	27
10^{-7}	3.6	4.1	3.9	38	78	58
10^{-6}	2.2	7.2	4.7	46	101	73
10^{-5}	2.9	8.1	5.5	11	20	16
Main effect of photoperiod	2.34	5.34		26	62	

Factors	Level of significance	LSD (0.05)	Level of significance	LSD (0.05)
Type of auxin	**	2.4	**	34
Photoperiod	***	1.7	***	24
Interaction	n.s.		n.s.	

n.s. Not significant; **$0.001 < p < 0.01$; ***$p < 0.001$.

concentration of 5×10^{-6} M proved to be the optimum for shoot proliferation and development in jackfruit.

Fresh and dry weights of shoots were significantly increased by longer photoperiods (12 and 16 h). Similar enhanced vegetative development has been reported for begonia [12]. The growth and development of shoots significantly increased with increasing temperature up to 30 °C which appeared to be optimal for jackfruit. While the shoot proliferation and growth was greater under longer photoperiods at 30 °C, it almost stopped at 35 °C and completely stopped at 40 °C in the presence of light, whereas in the dark proliferation continued up to 35 °C. This may be because at high temperatures respiration continues to increase, whereas photosynthesis begins to decrease [4], leading to the decline in shoot growth in the light under high temperature. Thus the results indicate that at optimum temperatures the plant growth is largely dependent on photoperiod. Beyond the optimum level the growth and development is not dependent on photoperiod, which is also true for begonia [1].

Among the various auxins, IBA and IAA were significantly more active than other auxins in initiating rooting in jackfruit. IBA produced significantly longer roots than IAA, possibly because IAA is broken down enzymatically in the light [2] and was thus unavailable to promote extension of roots. Increasing the concentration of IBA from 10^{-7} to 10^{-5} M increased

the number of roots, but the length was significantly decreased with the highest concentration. Such inhibition of root development by high concentrations of auxin may be due to the enhancement of ethylene biosynthesis in the root tissues [14].

It is now possible to recommend a method for tissue culture propagation of seedling jackfruit using nodal explants grown on 1/2 MS medium with BAP at 5×10^{-6} M for the shoot proliferation stage and IBA at 10^{-6} M for rooting the shootlets. Proliferation of shoots and rooting was very successful which suggests that modifications of the method could lead to propagation from mature tissues.

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References

1. Djurhuus R (1985) The effect of photoperiod and temperature on growth and development of *Begonia* × *tuberhybrida* 'Karelsk Jomfru'. Scientia Horticulturae 27: 123–131
2. Galston AW (1950) Riboflavin, light and growth of plants. Science 111: 619–624
3. Howard AL (1951) A manual of the timber of the world. 3rd edn. MacMillan, London
4. Kramer PJ, Kozlowski TT (1979) Physiology of woody plants. Academic Press, New York
5. Lane WD (1978) Regeneration of apple plants from shoot meristem tips. Plant Science Letters 13: 281–285
6. Lundergan CA, Janick J (1980) Regulation of apple shoot proliferation and growth in vitro. Horticultural Research 20: 19–24
7. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15: 473–497
8. Naik KC (1949) South-Indian fruits growing in India. 2nd edn. pp. 434–437. D.B. Taraporevala Sons Co., Bombay
9. Rahman MA (1986) Propagation of jackfruit (*Artocarpus heterophyllus* Lam.) by tissue culture and by cuttings. Ph.D. thesis, University of London
10. Rao NK, Narayanaswamy S, Chacko EK, Doreswamy R (1981) Tissue culture of the jack tree. Current Science 50: 310–312
11. Row-Dutton P (1976) *Artocarpus heterophyllus* jackfruit. In: RJ Garner, SA Chaudhri (Eds) The propagation of tropical fruit trees. pp. 269–290. FAO, CAB, London
12. Sandved G (1969) Flowering in *Begonia* × *hiemalis* Fotsch. as affected by day length and temperature. Acta Horticulturae 14: 61–63
13. Streets RJ (1962) Exotic forest trees in the British Commonwealth. Clarendon Press, Oxford
14. Wareing PF, Phillips IDJ (1981) Growth and differentiation in plants. 3rd edn. Pergamon Press UK