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INFLUENCE OF EXPLANT SOURCE ON THE *IN VITRO* PROPAGATION OF JACK (*Artocarpus heterophyllus* Lam.)

K. Rajmohan[1] and N. Mohanakumaran[1]
College of Horticulture, Vallanikksra 680 654, Trichur, India

One of the major problems in the micropropagation of woody perennials is maturity barrier. During the maturation process of the plant, several physiological changes take place that influence the *in vitro* behaviour of the explants. Such influence has been demonstrated in the ability of the explants to form adventitious and axillary buds, in the rate of shoot elongation and in the extent of rooting. It is important to select the most juvenile explants, as within the trees, there are some tissues in which juvenility is better maintained than in the others (Bonga, 1982). Tissues can be rejuvenated or at least invigourated by using auxins, cytokinins and gibberellins (Bonga, 1981) or by a series of selected treatments (Gupta *et al.*, 1981).

This paper describes the *in vitro* propagation of jack, as influenced by the source of the explants.

Materials and Methods

Shoot apices (about 1 cm long) from two-month old seedlings, six-month old grafts and fresh stem-sprouts of five, ten and thirty-year old trees were used as the explants.

The shoot apices were dipped in 95 per cent ethyl alcohol for 10 s and thoroughly washed with sterile water. Surface sterilisation was done by keeping them in 2 per cent sodium hypochlorite solution for 3 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 45 min and kept in sterile water at 4-5 °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 + GA_3 1 mg/l + activated charcoal 1 %) in darkness for four weeks with repeated subculturing. The cultures were then exposed to light for two weeks, after which they were inoculated into the different media.

The MS medium (Murashige and Skoog, 1962) was used as the basal medium. The concentration of BA in the proliferation medium (MS + BA 5 mg/l + NAA 0.2 mg/l + sucrose 30 g/l + agar 8 g/l + insoluble PVP 500 mg/l) standardised for five year old trees (Rajmohan and Mohanakumaran,

[1] Present address : College of Agriculture, Vellayani 695 522, Trivandrum, India

1983) was altered (5.0, 7.5, 10.0, 12.5, 20.0 and 40.0 mg/l) in order to determine the optima for the enhanced release of axillary buds from the explants of various sources.

Shoots from the proliferation medium were transferred after five weeks to an elongation medium (MS + BA 2.0 mg/l + NAA 0.2 mg/l + insoluble PVP 500 mg/l). The shoots were then cultured on MS medium containing activated charcoal (1%) for two weeks.

In vitro rooting of jack shoot cultures from various explant sources was attempted with MS medium (having half concentration of mineral salts) supplemented with various auxins (IBA, NAA, IAA and 2,4-D) either alone or in combination. The pH of the madia 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 26 + 2°C with a 16 h photoperiod (1000 lux) supplied by cool white flourescent tubes. The cultures for the rooting experiment were incubated in the dark.

Results and Discussion

Physiological age of th9 explants exhibited significant influence on the *in vitro* shoot proliferation and rooting of jack. The response was maximum for the seedling explants, followed by the explants of fresh stem-sprouts from five-year old trees. A drastic reduction was apparent in the response of the explants from fresh stem-sprouts of ten and thirty-year old trees, and of the explants from six-month old grafts.

In the case of seedling explants, maximum shoot proliferation (17.4 x) was achieved at BA 10 mg/l. For the explants from five-year old trees, the maximum number of fairly elongated shoots was recorded by BA at 5 mg/l (Table 1). Highly compressed and small shoots increased with the increase in the level of BA. Multiplication of the shoots in the case of explants from ten-year old trees was observed only at the lowest level (5 mg/l) of BA tried (Table 1). The rate of multiplication was very low (2.8 shoots per culture). The higher levels restricted the growth of the shoots resulting in 1.0 shoot per culture. At BA 5 mg/l, the length of the shoot was 2.26 cm and the longest leaf measured 2.52 cm. In the case of thirty-year old trees, multiple shoots from the explants were obtained only at BA at 5 mg/l (2.09 per culture). The shoot and the leaf were 1.97 cm and 1.32 cm long. Shoots turned brown and died ot the higher levels of BA tried (10, 20 and 40 mg/l), except at 7.5 mg/l (Table 1). The treatments were not effective in inducing multiplication of the shoot apices from six-month old grafts, although 100 per cent survival of the shoots and fairly satisfactory shoot growth were observed (Table 1).

While a multiplication rate of 17.4x was obtained for the seedling explants, the rates were only 4.50x, 2.80x and 2.09x for the explants from fresh stem-sprouts of five, ten and thirty-year old trees, respectively. In spite of taking the explants

from fresh basal sprouts, adopting suitable pre-culture treatments (like shaking to remove the endogenous inhibitors present, using activated charcoal and insoluble PVP to reduce the problem of oxidation of polyphenols, supplementing the culture medium with exogenous growth substances) and adopting serial subculturing, only limited response of the explants resulted. The use of explants from young grafts also did not help to make the response appreciable. Explants from mature trees are known to have "residual memory" making the micropropagation of woody parennials difficult (Durzan, 1984). Detailed investigations are necessary to understand the physiological and biochemical bases favouring juvenility so as to evolve suitable techniques to bring about rejuvenation and commercially acceptable degree of response.

In the case of seedling explants, excellent rooting of shoots (100% with 4.25 to 6.00 roots per shoot) was obtained in the treatments with IBA/NAA combinations 0.2+0.5 mg/l and 0.4+0.4 mg/l as well as with IBA 0.2 mg/l and 0.8 mg/l (Table 2). The IBA/NAA combination of 0.4+0.4 mg/l recorded the maximum number of roots (6.0 in 20.75 days). As for the *in vitro* rooting of the shoots from the cultures of the explants from five-year old trees, the treatment "half MS + IBA/NAA combination 2.0+2.0 mg/l for six-days and then transferring to half MS without growth substances" was found to be the most favourable, giving 70 per cent root initiation with 5.43 roots formed in 13.43 days (Table 2). *In vitro* rooting of shoots in the case of ten and thirty-year old trees and six-month old grafts was attempted with "half MS+IBA 1.6 mg/l + NAA 0.4 mg/l" and "half MS + IBA 2.0 mg/l + NAA 2.0 mg/l for 6 days and then transferring to half MS without growth substances" (Table 2). In the case of ten-year old trees, 40 per cent rooting was obtained in both the treatments with 2.5 roots per shoot. However,

Table 1

Effect of BA on the *in vitro* multiplication rate of jack shoots of different age groups
Basal medium: MS + NAA 0.2 ppm

Treatment (ppm)	Shoots per explant				
	Seedlings*	5-year** old trees	10-year* old trees	30-year old trees	6-month* old grafts
BA 5.0	8. 8	4.50	2.80	2.09***	1.00
BA 7.5	11.8	8.75	1.00	1.00**	1.00
BA 10.0	17.4	7.75	1.00	0	1.00
BA 12.5	—	—	1.00	0	1.00
BA 20.0	14.0	5.75	0	0	—
BA 40.0	13.8	0	0	0	—

* Average of five observations
** Average of four observations
*** Average of 11 observations
0 No response/explant died
— No treatment

Table 2

Effect of auxins on the *in vitro* rooting of jack shoot cultures from various age groups
Basal medium: Half the concentration of MS inorganic salts and full concentration of MS organic growth factors

Treatment (ppm)					Seedlings			5-year old trees		
					Root[a] initiation (%)	Roots per explant	Days for root initiation	Root initiation (%)	Roots per explant	Days for root initiation
1	IBA	0.2			100	4.25	28.00	—	—	—
2	IBA	0.8			100	5.00	29.25	—	—	—
3	IBA	1.6			75	7.33	29.00	—	—	—
4	IBA	8.0			0	0	0	0	0	0
5	NAA	1.0			0	0	0	0	0	0
6	NAA	2.0			—	—	—	0	0	0
7	IAA	10.0			0	0	0	0	0	0
8	2,4-D	0.1			0	0	0	0	0	0
9	NAA	0.05	+	IBA 0.4	0	0	0	0	0	0
10	NAA	0.4	+	IBA 0.2	—	—	—	0	0	0
11	NAA	0.4	+	IBA 0.4	100	6.00	20.75	0	0	0
12	NAA	0.4	+	IBA 0.8	—	—	—	0	0	0
13	NAA	0.4	+	IBA 1.6	—	—	—	80.0[b]	4.75	24.67
14	NAA	0.5	+	IBA 2.0	100	5.75	46.25	0	0	0
15	NAA	2.0	+	IBA 0.4	75	2.67	38.33	0	0	0
16	NAA (for 6 days and then without plant growth substances)	2.0	+	IBA 2.0	—	—	—	70.0°	5.43	13.43
17	IAA	0.1	+	IBA 0.1	50	2.50	32.50	0	0	0

a Average of four observations
b Average of 15 observations
c Average of 10 observations
0 No response
— No treatment

the latter treatment took only 24 days for root initiation, while the former took 44 days. When explants from thirty-year old trees were used, rooting was not observed in the former case, even after three subcultures. The latter treatment produced 15 per cent rooting after 46.7 days, with two to three subcultures. In the case of graft explants rooting was observed only in the "half MS + IBA/NAA combination 1.6 + 0.4 mg/l". The rooting percentage, after one subculture was 50. Only two roots per shoot were produced after 20.5 days.

The seedling explants responded to a wide range of auxin treatment for *in vitro* rooting. Response of the explants from five-year old trees was satisfactory,

recording 70 per cent root initiation with 5.43 roots in, 13.43 days at the best treatment. Response of the explants from ten and thirty-year old trees and young grafts was poor even after serial subculturing. Physiological and biochemical changes associated with the developmental age of the source of explant (Bonga, 1982) may be the reason for the reduced response observed. Use of fresh basal sprouts of the trees seems to have effected only a partial rejuvenation. The process of subculturing is reported to change the physiological state of the explants and gradually rejuvenate them (Vietiez *el al.*, 1935) to make *in vitro* root induction progressively easier. In the present study, the use of young grafts as a source of explants did not yield increased response as against the findings of Nantois (1980) in *Pseudotsuga menziesii*. In jack, grafting does not seem to bring about rejuvenation to a level favouring increase in response.

Table 2 (continued)

Treatment	10—year old tree			30—year old tree			6-month old grafts		
	Root initiation (%)	Roots per explant	Days for rooting	Root initiation (%)	Roots per explant	Days for rooting	Root initiation (%)	Roots per explant	Days for rooting
1	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—	—
13	40.0[a]	2.50	44.00	0	0	0	0	0	0
14	—	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—	—
16	40.0	2.50	24.00	15.00[b]	1.0	46.70	50.00[c]	2.00	20.50
17	—	—	—	—	—	—	—	—	—

a Average of five observations
b Average of 20 observations
c Average of four observations
0 No response
— No treatment

Summary

Physiological ago of the explants exhibited significant influence on the *in vitro* propagation of jack. Shoot apices from the seedlings registered a multiplication rate of 17.4x, with 100 percent rooting and 6.0 roots formed in 20.75

days. Explants from fresh stem-sprouts of five, ten and thirty-year old trees recorded shoot multiplication rates of 4.50x, 2.80x and 2.09x, respectively in five weeks. The corresponding rooting percentages were 70 (with 5.43 roots formed in 13.43 days), 40 (with 2.59 roots formed in 24 days) and 15 (with 1.0 root formed in 46.7 days) after two to three subcultures. Explants from six-month old jack grafts failed to produce multiple shoots; but exhibited 50 per cent rooting with 2.0 roots formed in 20.5 days.

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