Journal of Tropical Agriculture 35 (1997) : 1-4

IN VITRO SHOOT REGENERATION FROM AXILLARY BUD CULTURES OF MALABAR WHITE PINE *(VATERIA INDICA* L.) THROUGH TISSUE CULTURE

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Abstract : Axillary buds collected from seedlings of *Veteria Mica* L., the recalcitrant Malabar white pine, were cultured under *in vitro* conditions with the objective of standardising the micropropagation technique for this species. A few media combinations consisting of full and half strength mineral salts of Murashige and Skoog (MS) as well as woody plant medium (WPM) have been identified to be capable of supporting culture establishment and bad break. Among these, the media containing half strength mineral salts of MS supplemented with organic nutrients and growth regulators alone supported elongation and continued growth of shoots. Shoots were 1-2 cm in height with 2-3 leaves in eight weeks under controlled conditions with 16 h photoperiod at a temperature of 27+2" C. The success obtained is the first of this kind in this species.

Key words : Bud culture, Malabar white pine, micropropagation, tissue culture, *Valeria indica.*

INTRODUCTION

Indian copal tree (*Vateria indica* L.), popularly known as Malabar white pine, is a large handsome evergreen tree belonging to the family Dipterocarpaceae and found in the evergreen forests of South India. It is a well known species for commercial plywood manufacture and extraction of white dammar. Because of the highly recalcitrant nature of the seeds (Huang and Villanueva, 1993), raising nursery and production of sufficient planting materials is a limiting factor for large scale plantation programme of this species.

Micropropagation using *in vitro* methods is attracting considerable attention for obtaining large number of genetically pure elite populations of forest tree species. In the present investigation, attempts were made to standardise the technique of micropropagation of *V. indica* under *in vitro* conditions with the objective of rapid clonal multiplication of selected trees and to overcome the problems associated with the recalcitrant habit of this species.

MATERIALS AND METHODS

Explants used in this study were axillary buds of *V. indica* collected from 3 to 15 months old seedlings. The source seedlings were maintained in glass house and given prophylactic fungicidal spraying with Bavistine 50 per cent and Indofil M-45 both at 0.3 per cent.

Stem segments of approximately 25 to 30 cm with 10 to 12 nodes were excised from the seedlings. The leaves were removed leaving 0.5 cm of the petiole and the stem segments were cut into nodal segments of about 1 cm in size. They were surface sterilized by immersing in 0.1 per cent mercuric chloride for 5 min and washed three times with sterile distilled water. Buds were cultured on full and half strength mineral salts of Murashige and Skoog (1962) medium (MS) as well as Woody Plant Medium (WPM)(Lloyd and McCown, 1980) .

To find out the optimum growth requirements of the explants, the media were supplemented with different combinations of cytokinins, namely, 6-furfuryl aminopurine (kinetin), 6-benzyl aminopurine (BA) and N (isopentenyl) adeninc (2ip) at three levels, 0.1, 2.0 and 3.0 ppm with auxins, namely, indole-3-acetic acid (IAA), naphthyl acetic acid (NAA), 2,4-dichlorophenoxy acetic acid (2,4-D) and indole butyric acid (IBA) at three levels, 0.1, 0.5 and 1.0 ppm. Other growth supplements tried in the media included cycocel (CCC), silver nitrate, casein hydrolysate, adenine sulphate, cobalt chloride and coconut water. A total of 310 media combinations were attempted during the present investigation. The medium contained 30 g l^{-1} sucrose as carbon source. The pH was adjusted to 5.8 and the medium was solidified by 0.7 per cent agar. Twenty ml each of the medium was distributed to 25 x 150 mm culture vials and sterilized by autoclaving at 15 psi for 20 min. One nodal

Table 1. Media combinations favourable for inducing bud break in axillary bud cultures of *Veteria indica*

Basal media	Supplements, ppm					Culture establishment %	Bud break %
	Kinetin	BA	2ip	NAA	IBA		
MS	1.0	-	-	0.1	-	35.7	21.4
	1.0	-	-	0.5	-	57.1	7.1
	2.0	-	-	-	-	85.7	28.6
	3.0	-	-	0.1	-	28.7	21.7
	-	2.0	-	0.1	-	64.3	21.4
	-	-	1.0	-	--	85.7	21.4
	-	-	1.0	0.5	-	64.3	21.4
½ MS	-	-	2.0	-	0.1	85.7	7.0
WPM	1.0	-	-	0.5	-	42.9	21.5
	2.0	-	-	0.1	-	28.6	21.0

Table 2. Growth response of axillary bud cultures of *Veteria indica* in various media

Media combinations	Bud break		Shoots		% cultures with leaf	No. of leaves	
	%	Days taken	Number	Length after one month, cm		Produced	Retained after two months
MS + 1.0 ppm kinetin + 0.1 ppm NAA	21.4	24	1	0.6	14.2	1.0	Nil
0.5 ppm + NAA	7.1	25	1	0.8	7.0	1.0	Nil
MS + 1.0 ppm 2ip	21.4	23	1	0.5	21.4	1.0	Nil
½ MS + 2 ppm 2ip + 0.1 ppm IBA	7.1	18	1	1.0	7.1	2.0	2.0

segment each was vertically placed in the tubes. At least 14 cultures were initiated in each treatment. The experiment was replicated twice. Cultures were maintained under fluorescent light (approximately 400 lux intensity) with 16 h photoperiod at 25+2° C.

RESULTS AND DISCUSSION

Among the various media combinations tried in this study, culture establishment / bud break was noticed in 73 combinations. Ten media among these were identified as relatively more favourable for inducing bud break in axillary buds of *V. indica* (Table 1).

Explants cultured on MS medium containing 2.0 ppm kinetin exhibited good culture establishment (85.7%) and bud break (28.6%). Combinations of kinetin with certain levels of auxins also induced bud break. The favourable effect of kinetin has been reported in other tree species like *Eucalyptus teretixcornis* and

E. globulus (Gupta and Mascarenhas, 1987). In both MS and WPM media, NAA was the best auxin supplement along with kinetin than IAA, IBA and 2, 4-D ha inducing bud break. Other auxins had very negligible effect. Of the different concentrations of NAA tried, 0.1 and 0.5 ppm were found to be most effective either alone or in combination with cytokinins in both the media.

Among the various cytokinins used in plant tissue culture, BA is the cheapest and one of the most effective (Aboel-nil, 1987; Rai and Chandra, 1989). Enhancement of bud break in media supplemented with different levels of BA has been reported earlier in various tree species like *Betula uber* (Vijayakumar, *et al.*, 1990)., *Faidherbia albida* (Ruredzo and Hanson, 1993) and *Caesalpinia pulcherrima* (Rohman *et al.*, 1993). In *Vateria indica*, however, BA was found to be less effective in inducing bud break. Supplementing BA alone to the medium did not exhibit any favourable influence on the axillary buds of this species. It was observed that auxin NAA enhanced the effect of BA in inducing bud break. Bud break and proliferation was noticed in MS medium supplemented with 2.0 ppm BA and 0.1 ppm NAA. Among the various combinations of BA and auxins tried, this was the only one which exhibited any favourable response in the *in vitro* cultures of *Vateria*.

The naturally occurring cytokinin, 2ip has been reported to be more effective than kinetin or BA in a number of species including *Rhododendron* (Anderson, 1975) and *Allium sativum* (Bhojwani, 1980a). In *Vateria indica* also some of the combinations of 2ip were found to induce culture establishment and bud break from axillary bud cultures. The response was seen when 2ip was used alone or in combination with auxins NAA or IBA in full as well as half strength MS media. Among these two auxins, NAA had better effect than IBA in combination with 2ip. There is earlier report of 2ip being not very effective in inducing bud break in hybrid willow (Bhojwani, 1980b). In the present study, however, 2ip is found to be more effective than kinetin with regard to its potential for inducing bud break in Malabar white pine.

The interaction of cytokinin and auxin, however, is more complex. Auxin added to the medium has already been reported to nullify the suppressive effect of high cytokinin content on axillary shoot growth (Lundergan and Janick, 1980). In the present study NAA has been found to be the best auxin to be supplemented with cytokinins for inducing bud break in both MS and WPM media. The synergistic effect of NAA along with cytokinins in bud break has also been reported earlier in tree species like *Santalum album* (Sita *et al.*, 1980) and *Syzygium cumini* (Yadav *el al.*, 1990).

Among the various media additives tried in *Valeria* bud cultures, cycocel (CCC) had less effect on bud break in half MS medium and silver nitrate had moderate effect in WPM. Casein hydrolysate, adenine sulphate, cobalt chloride and coconut water were the other additives tried and found to lack any beneficial effects.

Among the combinations identified for bud break in *Valeria indica*, half MS supplemented with 2ip 2.0 ppm and IBA 0.1 ppm alone produced good shoots with normal leaves (Table 2). The average shoot length was 1.0 cm with two expanded leaves, eight weeks after culture. Lower level of 2ip (1.0 ppm) in combination with any of the three levels of IBA, namely, 0.1, 0.5 and 1.0 ppm tried in half MS failed to induce bud break. Higher concentrations of 2ip (3.0 ppm) in combination with any of the three levels of IBA also failed to induce bud break even with good culture establishment.

Cytokinin, kinetin or BA alone or in combination with auxin IBA did not favour healthy shoot formation even though bud break was obtained in some of these combinations. Kinetin (1.0 ppm) with 0.1 and 0.5 ppm IAA could induce shoot growth and leaf morphogenesis. However, further growth of the shoot was arrested and the leaves were also not retained for long. In this investigation, 2ip could be rated better than BA and next to kinetin with regard to its potential for inducing shoots in Malabar white pine. The action of 2ip might be stronger than kinetin or BA with other auxin combinations in half MS since this

was found to be more favourable for shoot growth and leaf retention. Similar result has been reported in *Kalmia latifolia* (Lloyd and McCown, 1980).

Even though a wide range of media compositions involving different basal composition, growth regulators and additives were tried, none was proved to be fully efficient. This is in agreement with the general recalcitrant nature of Dipterocarps to *in vitro* propagation. A media composition with perfect repeatability could not be identified in this investigation. One of the major hurdles in formulating a viable protocol in *V. indica* was the loss of morphogenetic potential of the buds with time. Inherent chemical compounds like phenols which may be present in the explants can be expected to exert growth inhibitory effects to the buds. Even though some media formulations could be identified with a good percentage of buds break and shoot differentiation, production of leaves and continued growth could be noticed only in few cases. It appears that further more regulated efforts need to be made for standardising the protocol for micropropagation of this species.

ACKNOWLEDGEMENT

This paper forms a part of the M.Sc. (Forestry) thesis of the senior author submitted to the Kerala Agricultural University, Vellanikkara during 1995.

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