In vitro culture of *Piper attenuatum* Ham. (Piperaceae): Callus induction and plant regeneration

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ABSTRACT

Callus induction and plant regeneration of *Piper attenuatum* was achieved from stem explants in Murashige and Skoog and Gamborg's basal media supplemented with 2,4-dichlorophenoxyacetic acid, indole-3-acetic acid, Kinetin, benzyladenine and palmyarh (*Borassus flabellifer*) endosperm extract. Root induction was obtained from stem explants in Murashige and Skoog medium supplemented with α-naphthaleneacetic acid, β-naphthoxyacetic acid, indole-3-acetic acid, benzyladenine, kinetin and coconut water. Shoot development was achieved in *in vitro* rooted stem segments planted in Murashige and Skoog's medium supplemented with indole-3-acetic acid, benzyladenine and also in indole-3-acetic acid and kinetin. The plantlets were successfully established in soil. This is the first report of *in vitro* culture of *P. attenuatum*.

Key words: micropropagation, palmyrah endosperm extract, *Piper attenuatum*.

Abbreviations

BA : Benzyladenine
B_5 : Gamborg's medium
CW : Coconut water
2,4-D : 2,4-Dichlorophenoxyacetic acid
IAA : Indole-3-acetic acid
IBA : Indole-3-butyric acid
Kn : Kinetin
MS : Murashige and Skoog medium
NAA : α-Naphthaleneacetic acid
NOA : β-Naphthoxyacetic acid
PEE : Palmyrah endosperm extract

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Piper attenuatum Ham. (Piperaceae), a species related to *P. nigrum* (black pepper) is a slender dioecious climber commonly seen in the forests of coastal Karnataka in India. The berries of the vine are used in Ayurveda and Unani medicines. Studies on micropropagation of this species was undertaken for its potential use in production of planting material, *in vitro* conservation of germplasm and crop improvement programmes.

Stem segments (nodal and internodal), leaf blade and petiole segments were used as explants. The explants were first washed in running tap water for 30 min and then in Tween-20 (polysorbate), surface sterilized with 0.1% mercuric chloride for 3-5 min and then thoroughly washed with sterile distilled water 3-4 times. The explants were then inoculated onto MS (Murashige and Skoog 1962) and B_5 (Gamborg, Miller & Ojima 1968) basal media supplemented with various growth adjuncts such as IAA ($1mgl^{-1}$), IBA ($1mgl^{-1}$), 2,4-D ($2mgl^{-1}$), NAA ($3mgl^{-1}$), NOA($1mgl^{-1}$), BA (0.5, 2, 3 and $10mgl^{-1}$), Kin (0.5 and 1.0 mgl^{-1}) and CW (0.5%). The pH of the culture media was adjusted to 5.7 and the cultures were maintained at 25±3°C under continuous illumination of approximately 1000 lux.

Though attempts were made to induce callusing, rooting, shooting and regeneration of plantlets from different explants, callus roots and plantlet regeneration was obtained only from stem segments. Stem segments (nodal and internodal) on MS medium supplemented with IAA ($1mgl^{-1}$) and BA ($10mgl^{-1}$) gave whitish shiny callus. Callusing was initiated at the amputed region (Fig.1) which later spread to the entire surface. The proliferation of the

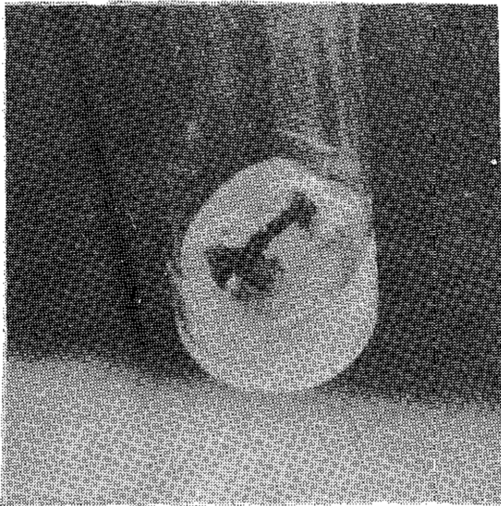

Fig. 1. Callus initiation from stem explant in *Piper attenuatum*

Fig.2. *In vitro* plantlet regeneration from stem callus of *Piper attenuatum*

Table 1. *In vitro* responses of *Piper attenuatum* on MS and B_5 basal media with different concentrations and combinations of auxins and cytokinins

Basal media	Explant	Growth regulator		Nature and per cent *in vitro* response (mgl^{-1})
		Auxin	Cytokinin (mgl^{-1})	
MS	Stem	IAA ($1 mgl^{-1}$)	BA ($10 mgl^{-1}$)	C^{+++};10/12* (83.3%)
	Stem	IAA ($1 mgl^{-1}$)	Kin ($1 mgl^{-1}$)	C^{++}; 8/12 (66.7%)
	Stem callus	IBA ($1 mgl^{-1}$)	BA ($3 mgl^{-1}$)	(Indirect plantlet regeneration) 5/12 (41.6%)
	Stem	IBA ($1 mgl^{-1}$)	BA ($10 mgl^{-1}$)	(Direct plantlet regeneration) 8/10 (80.0%)
	Stem	IAA ($1 mgl^{-1}$)	BA ($2 mgl^{-1}$)+ PEE (0.5%)	(Direct plantlet regeneration) 5/11 (45.5%)
	Stem	NAA ($3 mgl^{-1}$)	BA ($0.5 mgl^{-1}$)	R^{+++}; 7/11 (63.6%)
	Petiole	IAA ($1 mgl^{-1}$)	BA ($10 mgl^{-1}$)	C^{+++}; 5/11 (45.5%)
	Leaf	IAA ($1 mgl^{-1}$)	BA ($10 mgl^{-1}$)	C^{+}; 3/12 (25.0%)
B_5	Stem	IAA ($1 mgl^{-1}$)	Kin ($1 mgl^{-1}$)	C^{++}; 7/12 (58.3%)
	Stem	NOA ($1 mgl^{-1}$)	CW (0.5%)	R^{++}; 5/11 (45.5%)
	Leaf	2,4-D ($2 mgl^{-1}$)	Kin ($0.5 mgl^{-1}$)	C^{+}; 3/10 (30.0%)

*No. of explants responded/Total no. of explants inoculated
C^{+++} Profuse callusing; C^{++} Moderate callusing; C^{+} Poor callusing (Slight deformation); R^{+++} Profuse rooting; R^{++} Moderate rooting

callus was slow and it took 40 days to cover a stem section of 2 cm size. MS media supplemented with $1 mgl^{-1}$ IBA and $3 mgl^{-1}$, was the best media for shoot bud differentiation and plant regeneration (Fig. 2) from stem callus. Callus was also obtained in stem section using MS as well as B_5 basal medium supplemented with IAA ($1 mgl^{-1}$), and Kin ($1 mgl^{-1}$). However, compared to first concentrations i.e., IAA ($1 mgl^{-1}$) and BA ($10 mgl^{-1}$), the proliferation of callus in these cases was slow. Leaf blade was least amenable to any response except for slight deformation of the surface (Table 1).

Direct regeneration of plantlets from nodal segments was obtained on MS medium supplemented with IBA ($1 mgl^{-1}$), BA ($10 mgl^{-1}$) and also in IAA ($1 mgl^{-1}$), BA ($2 mg l^{-1}$) and PEE (0.5%).

B_5 medium supplemented with IAA ($1 mgl^{-1}$) and Kin ($1 mgl^{-1}$) promoted bud development into 2-3 shoots with very little or no callus in 60 per cent of the cultures. Root initiation later took place in B_5 medium supplemented with NOA

(1mgl^{-1}) and CW (0.5%) and the explant developed into a plantlet. Root induction was also achieved on stem segments inoculated onto MS medium supplemented with NAA (3 mgl^{-1}) and BA (0.5 mgl^{-1}). The plantlets obtained were healthy and attained a height of 3-5 cm in 60-70 days and were successfully transfered to garden soil with 66.66 per cent survival.

The results of the present study demonstrates that *P. attenuatum* is amenable to *in vitro* culture techniques. Although a number of other species of *Piper* have been aseptically cultured (Aminuddin *et al.* 1993; Bhat, Kackar and Chandel 1992; Sarasan *et al*. 1993), *P. attenuatum* has been cultured *in vitro* for the first time. We have also not come across any reference so far on the use of palmyrah (*Borassus flabellifer* L.) endosperm extracts as an adjunctive for *in vitro* culture studies.

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