Micropropagation of betel vine (*Piper betle* L.)

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ABSTRACT

Betel vine (*Piper betle* L.) cv. Lakkuvalli was successfully micropropagated on Woody Plant Medium (WPM). Different explants from shoot, leaf and root tissues developed multiple shoots and rengenerated into plantlets either directly or through intervening callus phase on WPM supplemented with 3 mgl^{-1} benzyladenine and 1 mgl^{-1} kinetin. The excised shoots developed good root system on growth regulator free medium of the same composition. The plantlets were transferred to soil with 80% success.

Key words : Micropropagation, *Piper betle* L.

Abbreviations

BA : N_6-benzyladenine
WPM : Woody Plant Medium (Mc Cown & Amos 1979)

Betel vine (*Piper betle* L., Piperaceae) is a perennial dioecious climber, probably native to Malaysia. It is cultivated extensively in India for its leaves which are masticatory. As a masticatory it is credited with digestive, stimulant and carminative properties. Medicinally it is useful in catorrhal and pulmonary afflictions (Anonymous 1969). In India it is grown in an area of around 40,000 ha. Betel vine is also an important crop in Bangladesh, Sri Lanka, Malaysia and Myanmar. The aim of the present study was to standardize protocols for micropropagation of *P. betle* by tissue culture.

Young shoot and leaf tissues were collected from field grown plants of cv. Lakkuvalli on bright sunny days and were washed in running water and later with detergent solution (teepol) for 20 min. They were surface sterilized with 0.1% mercuric chloride solution for 5 min. and washed in 3-4 changes of sterile water. The surface sterilized shoots (1-2 cm long) and tender leaves (either whole or portions) were inoculated in the culture medium under aseptic conditions. The basal medium used was that of Woody Plant Medium (Mc Cowen & Amos 1979) suplemented with BA (0.5, 1.0, 2.0 mgl^{-1}) and kinetin (0.5, 1.0

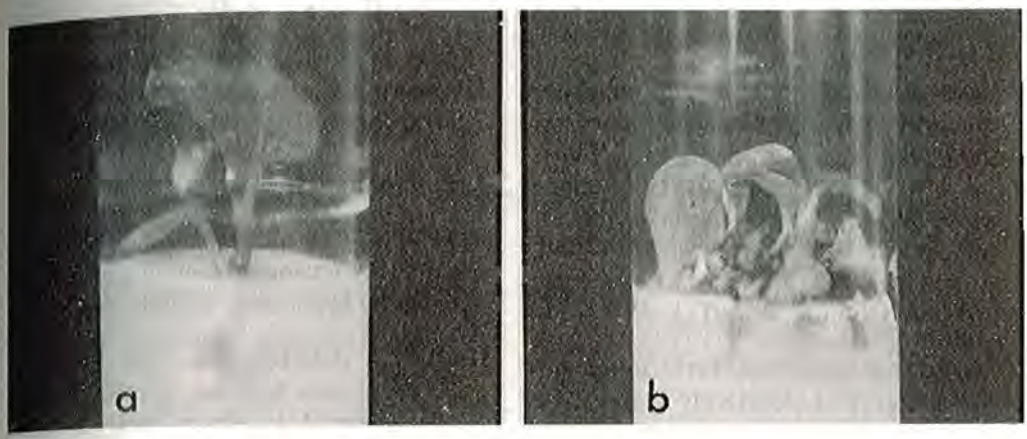

Fig. 1. Micropropagation of *Piper betle* L.
a. Production of multiple shoots at the base of shoot tip explant b. Plant regeneration from leaf derived callus.

and 2.0 mgl^{-1}) in various combinations. The pH of the medium was adjusted to 5.8 before autoclaving at 1 kg/cm^2 pressure (121°C) for 20 min. The medium was solidified with 0.7% bacteriological grade agar. All the cultures were incubated at 25 + 2°C with 14 h photoperiod provided by cool fluorescent tubes giving a light intensity of 30 μ mol^{s-1} m^{-2}.

All the explants responded to the various combinations of WPM tested. Callus production and shoot regeneration was noticed to a certain extent in all the combinations except growth regulator free medium where there was only rhizogenesis. However, WPM supplemented with 3 mgl^{-1} BA and 1 mgl^{-1} kinetin was the best for both multiple shoot production and regeneration of plantlets. The shoot tissues, both shoot tips and internodal segments, when cultured on this medium developed multiple shoots directly with very little or no callus in 50% of the cultures (Fig. 1a) In the rest of the cultures there was callus development and plant regeneration from the callus by organogenesis. The number of plants regenerated ranged from 5-10. Tender leaves with a portion of the petiole intact were better for plant regeneration. They gave rise to 10-20 plantlets by direct organogenesis (shoot formation) in 65% of cultures. Plant regeneration was mostly from the petiolar end. In the rest of the cultures there was initial callus development which subsequenty gave rise to plantlets by organogenesis (Fig. 1b). The elongated shoots could be excised after 50-70 days of culture and transferred to rooting media. The shoot tips collected either from field grown plants or from *in vitro* culture shoots developed good root system, within 40 days, when cultured on WPM devoid of growth regulators.

When rooted plantlets were cultured on WPM with 3 mgl^{-1} BA and 1 mgl^{-1} kinetin, there was callus development to a certain extent from all the tissues ie., roots, leaves, which were in contact with the medium, which later gave rise to adventitious shoots. The well rooted plantlets were transferred to soil (top

soil, sand and vermiculite in equal proportions) with 80% success when placed in humid chamber for first 20 days.

This is the first report on micropropagation of *P. betle*. The earlier reports on micropropagation of the genus *Piper* were confined to two other important species viz., *P. nigrum* (Broome & Zimmermam 1978; Mathew & Rao 1984; Philip *et al.* 1992) and *P. longum* (Bhat, Kachar & Chandel 1992).

The micropropagation techniques developed could be effectively utilised for production of disease free clonal planting material, production of somaclones and exploitation of soma-clonal variation in crop improvement.

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