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Short Communication

MICROPROPAGATION OF AN ENDANGERED SPECIES OF *PIPER BARBERI* GAMBLE AND ITS CONSERVATION

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The genus *Piper* is represented by about 13 species in South India of which *Piper barberi* is very rare (Gamble, 1925). The other important species of *Piper* and *P. nigrum* (black pepper), *P. betle* (betel vine) and *P. longum* (Indian long pepper). *Piper barberi* is reported to be almost extinct and recorded in the red data book of Indian plants (Nayar and Sastry, 1988). After the type collection of C.A. Barber in 1901, it was thought to be extinct. Its occurance was again repoted six decades later by Dubramanyam and Henry (1970) and later by Nirmal Babu *et al.*, (1992) and Mathew and Mathew (1992). *Piper barberi* is a remarkable species and can be easily distinguished by its pinnately veined leaves and spikes borne on very long filiform peduncles. This species is a slender deiocious climber and has little resemblance with South Indian taxa of *Piper*. It is more alike to Central and Northern South American forms with its reticulately veined leaves, persistant prophylls and long peduncles (Fig. 1A), and at the same time differs from them in its climbing habit and dioecious nature. Probably *P. barberi* could be sole survivor of an ancestral type that reached India from Central American region (Nirmal Babu *et al.*, 1992). The present study was taken up to standardize protcols for micropropagation for rapid multiplication and conservation of this endangered species.

Shoot tips, approximately 2-3 cm long, were excised from field grown plants and were washed in detergent (teepol) for 15 minutes. They were surface sterilized with 0.1% $HgCL_2$ for 5 minutes and were rinsed in 3 times in sterile water and transferred to culture media in aseptic condition. Fully expanded, Woody Plant Medium (McCown and Amos, 1979) was used as basal medium with 2% sucrose and 0.6% agar supplemented with N^6 benzyladenine (BA; 0.5, 1.0, 2.0 and 3.0 mgl^{-1}) and kinetin (0.5, 1.0 mgl^{-1}) in

various combinations. The pH of the medium was adjusted to 5.8 before autoclaving at 1kg cm^{-2} pressure (121° C) for 20 minutes. All the cultures were incubated at 22 ± 2°C with 14 h photoperiod of 30 μ mol s^{-1} m^{-2} light intensity provided by cool fluorescent tubes.

Establishment of cultures from shoot explants taken from field grown plants were difficult due to contamination by endogenous bacteria. About 40% of the cultures could be established. Further explants were obtained from 3-4 months old *in vitro*-cultured shoots. Different explants *viz.*, shoot tips, nodal segments and leaves were cultured on WPM supplemented with various concentrations of BA and kinetin as mentioned above. The best response was obtained on WPM supplemented with 3 mgl^{-1} BA and 1 mg 1^{-1} kinetin for the production of multiple shoots while growth regulator-free medium was adjudged better for rooting. Hence, these two media (growth regulator free WPM and WPM with 3mgl^{-1} BA and 1mgl^{-1} kinetin) were used for further studies.

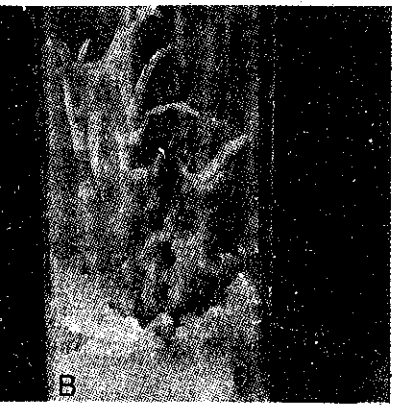
An initial swelling at the base of the shoots in 45% of the cultures in both shoot tip as well as nodal segment explants on WPM supplemented with 3 mgl^{-1} BA +1 mgl^{-1} kinetin was observed. Multiple shoots were observed from the base as well as from the nodal regions in over 90% of the cultures within 60 days of culture on above medium. The number of multiple shoots ranged from 10 to 20 (Fig. 1B). Most of the shoots developed to 3-5 cm in length in about 90 days of culture, however, they did not develop roots on the same medium.

When whole leaves, with a portion of the petiole, collected from *in vitro* grown plants were cultured on the WPM + 3 mgl^{-1} BA + 1mgl^{-1} kinetin direct regeneration of plantlets from the cut end of the petiole in over 75% of the cultures was observed. The number of plants regenerated ranged from 5 to 15 in 60 days of culture (Fig. 1C).

On growth regulator-free WPM, rooting was induced in over 90% of shoot cultures obtained from both shoot and leaf explants. Over 5 cm long roots developed ranging from 3 to 6 in about 40 days from culture on root inducing medium.

The *in-vitro*-raised rooted plantlets were transferred to potted soil mixture (garden soil : Perlite: sand; 1:1:1) and hardened in humid chamber (80-90% RH) for 20-30 days. Over 80% plants survived in pots (Fig. 1E).

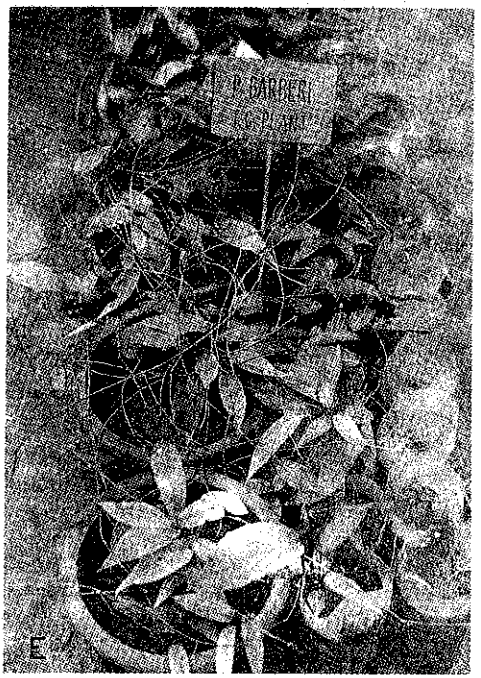
Studies have shown that *P. barberi* cultures could be maintained in *in vitro* repositories (Fig. 1D) by yearly sub-culture on minimal growth medium i.e. on WPM supplemented with 25 gl^{-1} sucrose and 5 gl^{-1} mannitol in sealed culture tubes.


B


A


C


D


E

Thus, *P. barberi* could be successfully micropropagated from both shoot and leaf explants, by direct regeneration using modified Woody Plant Medium. This is the first report on micropropagation of *P. barberi*. By using this protocol over 200 micropropagated plants were planted in the pots. These plants can be conserved either in field repositories or may be re-introduced into their natural habitat. Being a vegetatively propagated species, it is also possible to conserve this species in *in vitro* repositories.

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