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PLANT REGENERATION FROM TISSUE CULTURES OF *PIPER COLUBRINUM* Link.

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ABSTRACT

Piper colubrinum **Link is a distant relative of the cultivated black pepper (*Piper nigrum* L.) and is the only species reported to be totally immune to *Phytophthora* foot rot of black pepper. This species can be successfully micropropagated from leaf, stem and root tissues on WPM as a basal medium supplemented with growth regulators. Shoot tip explants developed excellent root system on hormone free medium and they developed multiple shoots on woody plant medium (WPM) supplemented with cytokinin. Plantlets can be regenerated from stem, leaf and root tissues by direct organogenesis as well as through callus phase on WPM + 3 $mg1^{-1}$ benzyladenine (BA) and 1 mgl^{-1} Kinetin. This tissue and callus culture protocols may help in attaining the ultimate objective of transfer of resistance from *P. colubrinum* to cultivated black pepper through genetic engineering.**

INTRODUCTION

Piper colubrinum Link - is a South American species of *Piper* and is distantly related to *Piper nigrum* L., the black pepper of commerce. The cultivation of black pepper is threatened by *Phytophthora* foot rot and all the cultivars are susceptible to this disease. Burrowing nematode (*Radopholus similis*) and pollu beetle (*Longitarus nigripennis*) are the other major pests limiting production. *Piper colubrinum* is immune to the foot rot disease and is highly resistant to the burrowing nematode and pollu beetle. Conventional hybridization between these two species to transfer resistance is not possible as the species are very distant and incompatible. Biotechnological means like somatic hybridization and gene transfer are more appropriate to develop resistant black pepper lines. For any such efforts development of plant regeneration protocols forms the basic requirement.

Grafting of *P. nigrum* on *P. colubrinum* root stock was another possibility where grafted plantlets may overcome nematode as well as *Phytophthora* foot rot problems. This requires large number of plantlets to be used as root stocks though there are a few earlier reports on micropropagation of two of the *Piper* species viz., *P. nigrum*, and *P. longum* (Broome and Zimmerman, 1978; Chua, 1981; Mathews and Rao, 1984; Philip *et. al.*, 1992; Bhat *et. al.*, 1992) and there are no reports on *P. colubrinum*. The authors report the successful standardization of plant regeneration protocols from mainly leaf and callus cultures of *P. colubrinum*.

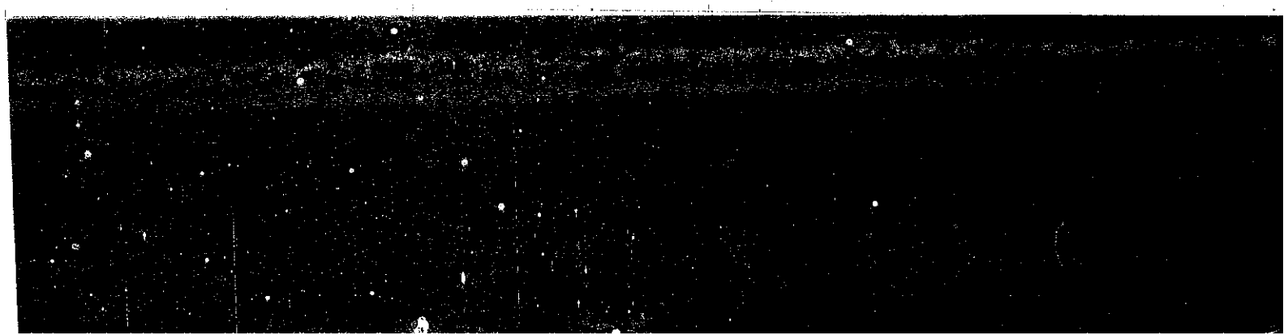
MATERIALS AND METHODS

Source of explant

Tender leaf and shoot explants were taken from field grown plants of *P. colubrinum* and washed in running water for 10 minutes. They were then dipped in 3% Bavistin for another 10 minutes and washed thoroughly in sterile water. These explants were trimmed and surface sterilized with 0.1% $HgCl_2$ for 5 to 10 minutes. This was followed by washing of the explants in 3 to 4 changes of sterile water. These surface sterilized shoot tips (1 to 2 cm long), leaf discs, and whole leaves with portion of petioles were inoculated in the culture medium in aseptic condition.

Culture medium and culture conditions

The WPM (Mc Cowen *et. al.*, 1979) was used as basal medium supplemented with 2, 4-D (0.5 and 1.5 mgl^{-1}). BA 0.5 and 1.5 mgl^{-1} and



NAA (0.5 and 1.5 mgl^{-1}) and Kinetin (1 mgl^{-1}) in various combinations. The pH of the media was adjusted to 5.8 before autoclaving at 1.0 kg/cm^2 pressure (121° C) for 20 minutes. The culture medium was gelled with 6.5 gml^{-1} of bacteriological grade agar (Hi Media). All the cultures were incubated at 25 ± 2° C with 14 hrs of photoperiod, produced by cool white fluorescent tubes with a light intensity of 30 mol m^{-2} s^{-1}.

RESULTS AND DISCUSSION

Establishment of cultures

Various explants viz., shoot tips, whole leaves with portion of petiole, portion of leaf lamina etc., were cultured on hall strength WPM. Contamination mainly due to endogenous bacteria was the major problem. When topmost segments of tender actively growing orthotropic shoots were used as explants about 50 to 60% cultures were free of contamination though in a few the bacterial contamination occurred again. Browning of tissues due to phenolic exudates was another problem. This was overcome by frequent transfer to fresh medium, in initial stages and subsequently such transfers were made in every 15 to 20 days.

In vitro responses

All explants responded readily to WPM supplemented with BA (0.5, 1.0, 2.0 mgl^{-1}). Callus production and shoot regeneration was noticed to certain extent in all the combinations of BA, Kinetin and 2, 4-D. However, 1/2 WPM with 3 mgl^{-1} BA and 1 mgl^{-1} Kinetin gave best results.

Shoot explants

When shoot tip explants were cultured on 1/2 WPM with 3 mgl^{-1} BA and 1 mgl^{-1} Kinetin, there was initial growth of hard and globular callus in 70% of the cultures. Within 20 to 25 days organogenesis with large number (20 to 150) of shoot primordia were obtained from the shoot callii. The shoot primordia developed into full grown shoots in most of the cases. In 30% of the cultures on WPM + 3 mgl^{-1} BA and 1 mgl^{-1} Kinetin direct organogenesis into shoot primordia without intervening culture phase was noticed. These primordia also developed into full fledged shoots in the media of same composition.

Excellent rooting of the shoots were obtained by transferring the shoots to hormones free media.

Leaf explants

The leaf explants were more responsive than shoot for organogenesis and plant regeneration, leaf explants developed callus from the cut end of 1/2 WPM with 3 mgl^{-1} BA and 1 mgl^{-1} Kinetin. In 30-45 days of culture on the same medium numerous shoot primordia developed from the callus. Direct and indirect morphogenesis was observed in the same media from the leaf explants. The shoot primordia, so developed, transferred into full-fledged shoots and these shoots got easily rooted in hormone free media.

Conversion of root meristem into shoot

When small shoot explants of *P. colubrinum* with two roots were transferred into WPM with 30 mgl^{-1} BA and 1 mgl^{-1} Kinetin. Swelling up of roots and formation of bulbil like structure were developed at the base of the roots in a period of 40-45 days. Conversion of these root meristem to shoot meristem took place in a period of another 35 days and shoots were developed from the bulbil like structures. Shoot development occurred throughout the swollen roots in certain cases. The same phenomenon was also observed in other species of piper viz., *P. longum* and *P. chaba*.

These plants could be easily established in soil after hardening.

The response of *P. colubrinum* was excellent in WPM. Leaf, stem and root explants of *P. colubrinum* responded readily for micropropagation. No growth regulators required for *in vitro* rooting of shoots. This excellent regeneration system of *P. colubrinum* could be made use of in transfer of resistance to *Phytophthora* to the cultivated species by biotechnological means.

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