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Original Research Article

Invitro Micropropagation of *Piper betle* L.

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Keywords

Piper betle L, Micro propagation, Callus, Growth regulators

A B S T R A C T

Plant tissue culture has provided new insights into plant biology and has become an important tool for the improvement of crop species. Recent progress made in tissue culture technique has indicated, the potential application of biotechnology in the improvement of many crop in herbs. From this study concluded that shoot tip and leaf base explants of *Piper betle* are ideal for establishing *in vitro* culture. MS basal medium supplemented with 1mg/l of IAA and 0.5mg/l BAP is suitable induction of multiple shoots in shoot tip and leaf base explants in *Piper betle*. Further works on induction of embryoids and development of hardening method need to be carried out for successful in vitro culture in this important Indian medicinal plant.

Introduction

Recently medicinal plants occupy an important place in health care, cosmetics a food industries throughout the world. Herbal drugs are more preferred than allopathic drugs because of higher efficiency affordability, easy availability and causing less or side effect. Even western world begin to use herbal drugs and herbal formulation described in traditional medicines like, chines medicines and traditional medicines like Aurveda and siddha literature for curing various diseases. Apart from traditional system of medicines, various Indian communities use many medicinal plants for therapeutic purpose and this unmodified system is called "Folk medicine" or Ethnomedicne.

In recent years plant biotechnology has made an impressive progress as one of the frontiers of biotechnology of scientific and economic importance. Development In the technology of plant tissue culture science the bioengineering experiments by White (1934 & 1937) Skoog and Murashige and Skoog (1962) have contributed in establishing a strong foundation for the application of this versatile technology. Important medicines are economical plants are becoming extinct and endangered due to heavy unscientific and unsustainable exploitation and low propagation response. Tissue culture methods valuable tools to propagate rapidly and to generative new varieties of medicinal plants.

Plant tissue culture has provide new insights into plant biology and has become an important tools for the improvement of crop species . Recent progress made in tissue culture technique has indicated, the potential

application of biotechnology in the improvement of many crop in herbs. Plant tissue culture technique has been successfully utilized o generating genetic variability for selecting better genotypes in many crops in many crops in plants. Herbs are staging a come.ack and an herbal renaissance is blooming across the world. They have been successfully utilized on generating genetic variability for selecting better genotype in improvement of many crop in plants. Herbs are staging a come back and an herbal renaissance is blooming across the world. they have been prized for their medicinal, flavoring and aromatic qualities for countries and yet for a while they were over's had owed by the synthetic products of the modern cultivation but once having realized their serious side effects people are going back to nature with hopes of safety and security.

Secondary metabolites from plants namely alkaloids, flavonoids, saponins and terpenes have played a vital role in pharmaceutical, cosmetic, perfumery, drying ad flavor industries. These drug ,flavor , essential, oils , and colors derived from plants have no apparent in plant primary metabolism but often play an adaptive role . Secondary metabolites are commercially feasible and as such for enhancing the *in vitro* production of natural products.

The present work deals an *in vitro* method for hairy root induction of Piper betel Linn. Herbal improvement through biotechnology means provides new hopes as success in improving them conventional breeding is limited due to their narrow genetic base and sexual incompatibility for the wild relatives.

Betel leaf is traditionally known to be useful for the treatment of various diseases like bad breath, boils and abscesses, conjunctivitis, constipation, headache, hysteria, itches, mastitis, leucorrhoea, otorrhoea, ring worm, swelling of gum, rheumatism, abrasion, cuts and injuries etc as folk medicine while the root is known for its female contraceptive effects (Chopra *et al.*, 1956: Khanra, 1997). The betle leaves really do not have any match as a cheap, natural and easily available appetizer, digestive , mild stimulant, aphrodisiac and refreshing mastication. Chewing of betel leaves produce a sense of well- being , increased alertness, sweating , salivation, hot sensation and energetic feeling with exhilaration. It also increases the capacity to exercise physical and mental functions more efficiently for a longer duration but it may produce a kind of psychoactive effect causing a conduction of mild addiction to habituation and withdrawal symptoms (chu,2001: and Jain,1996).

Materials and Methods

Collection of Plant

The plant source piper betel (L.) (piperaceae),commonly known as Indian betle leaves was collected from Thiruvaiyaru at Thanjavur Dt, India.

Methodology

Selection of Explants

Leaf explants and apices were used for the present study. The first fully expanded leaves in the shoot apex were collected from the garden grown plant. The explants were excised with the help of sterile forceps and blade. The nodes were cut in to 0.5-1.0 cm sized segments and care was taken that each explants include the midrib portion. Apical shoot buds measuring 10-15mm in length with 2-3 lea primordial attached were also used. The selected explants were sterilized by 0.1% of Mercury Choloride.

Preparation of Medium

Murashige and skoog's (1962) (MS) medium was prepared with different growth regulators such as IAA, BAP, 2,4 D and NAA at various concentrations. Regular observation at an interval of two days was made for the formation of callus, change of colour and initiation of the root and shoot .

Results and Discussion

Different explants like leaf base, leaf lamina, node and shoot tip of piper betle were cultured on different concentration of hormones (IAA,IBA,2,4-D, BAP kinetin). It was observed that all explants showed growth response like enlargement, ignition of callus and formation of shoot and root. therefore all the above explants of piper betle were used throughout the present study.

Effect of different auxins and cytokinins on growth of different explants of piper betle were observed and given in I-V.

Out of different concentrations (0.5-2.0 mg/l) of IAA used after 4 weeks of culture , maximum growth of all the the explants was observed on 1mg/l followed by 0.5mg/l . Of all the explants registered the highest growth (925.80 mg fr.wt) followed by leaf base , at I mgh/l concentration of IAA. Nodal explants showed the least growth increments in all IAA concentrations. Shoot tip and leaf base explants registered more than twenty four fold growth increment in fresh weight and dry weight basis on MS medium supplemented with1mg/IAA callus or morphogenesis were not observed.

When IBA was supplemented in different concentrations to MS medium, all the explants showed maximum growth increment at 1mg/l conc. Among explants, shoot tip explants registered the highest growth (898 mg fr.wt.0 1mg/1 IBA conc. Followed by leaf base explants showed more than twenty four, fold growth increment after, 4 weeks culture period, no callus or morphogenesis observed in all the concentration of IBA.

2,4-D at different concentrations induced the highest growth increment and formation of callus in almost all the explants (table- III). Callus was initiated in all the explants after 2 weeks in culture. Shoot tip explants were more proliferate and registered the highest growth 919519 mg fr.wt.) at 1.5mg/l 2,4-d concentration. At this concentration all other explants also showed a maximum growth.MS medium supplemented with 1mg/l 2,4 –D also induced good growth on all the explants. Nodal explants showed the least growth in all the 2,4-D concentrations.

The different concentration BAP induced more growth, compared to other auxins and cytokinins such as IAA,IBA,2,4-D and kinetin. Profuse growth (1009mg fr.wt.) was induced by the 0.5 mg/l of BAP in shoot tip explants. At the above concentrations of BAP, nodal explants at different concentration of BAP was in the following order 0.5>1.0>1.5>2.0 (table-iv). In this medium also, shoot tip and leaf base explants showed the best growth response.

Next to BAP, kinetin induced more growth in all the explants, maximum amount of growth (984 and 890 mg fr.wt) produced at the conc.0.5mg/l and 1.0 mg /l at shoot tip explants (Table V). The least growth was obtained by the nodal explants. The growth gradually decreased as the concentration of kinetin increased.

The organogenetic pattern of different explants (lamina, leaf base, node and shoot tip) on MS medium supplemented with different concentrations and combinations of auxins and cytokinins was observed. In

lamina explants cultured on MS medium with BAP 0.5mg/l induced callus (fig.2) and BAP 2mg/l + 2,4-D 1.5 mg/l produced callus, shoot and root and the frequency was 90,71 and 90% respectively (Table – VI: Fig: 3-5).

The nodal explants produced the least amount of growth. The medium with BAP 1mg/l +2,4-D 1.5mg/l induced white and friable callus in nodal explants (Fig:6). Induction of multiples shoots are absent in nodal explants, cultured containing medium BAP 1.5mg/L+2,4-D 2mg (Fig:8)

In leaf base explants inoculated on medium with BAP 0.5mg/l+IAA 1mg/l and BAP 1.5mg/l+2,4-D 2mg /l produced multiple shoots only. Which frequency was 95,90 and 89%of the leaf base and 92,95 and 61% respectively(Fig. 7&9).

Fig.1 Piper Betle L.

Callus, Shoot and Root Induction of Sample

Fig-**2 Medium: MS + BAP 0.5 mg /l +IAA 1.5mg /l.**
Fig-4 Medium : MS + BAP 2.0 mg/l + 2,4-D 1.5mg/l ,
Fig-5 Medium : MS +BAP 2.0 mg/l+ 2, 4-D 1.5mg/l.
Fig -7 Medium: MS + Bap 0.5 mg/l +IAA -1.0MG/L
Fig-8 Medium : MS + BAP- 1.5 mg/l + IAA-2.0mg/l.
Fig-9 Medium: MS+ BAP-1.5 mg/L +2,4-D-2.0 mg/L

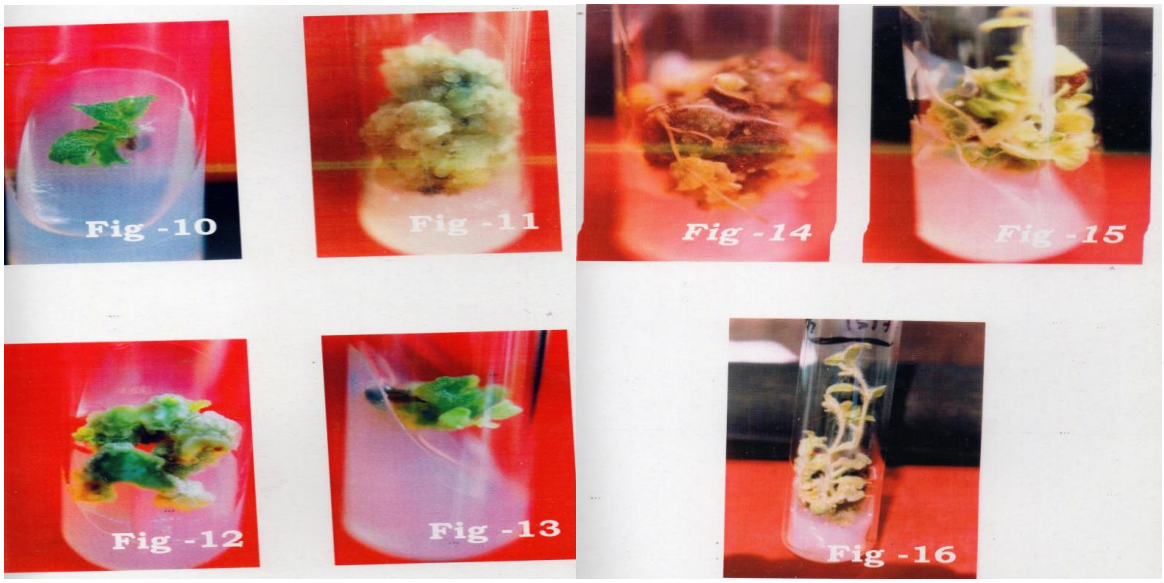

Fig- 10 Medium: Ms +bap-0.5 mg/l +2,4-D-0.5 mg/l
Fig-11 Medium MS +BAP 0.5 mg / 1=2-4 D1.5mg/l
Fig-12 Medium: MS+Bap 0.5 mg/l+IAA 0.5mg /l
Fig -13 Medium: MS+BAP0.5 mg /l+IAa 0.5mg/l.
Fig -14 Medium:MS + BAP 0.5 mg/L +IAA 1.0 mg/l
Fig-15 Medium; MS+Bap 0.5 mg/l =IAA1.0mg/l
Fig-16 Medium: MS + BAP 0.5 mg/l +IAA 1.0mg/l

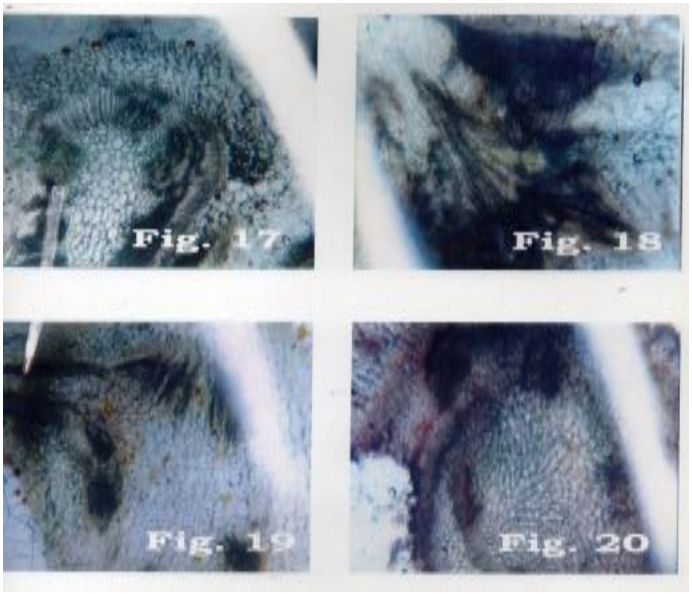

Fig-17 C.S. of shoot forming callus showing vasculature to new shoots
Fig-18 C.S of multiple shoot Forming callus showing braching vasculature to new shoots
Fig-19 C.S of nodal region from field grown plant showing oil giobules
Fig- 20 C.S of nodal region from in vitro plantlet showing absence of oil globules

Shoot tip and leaf base of piper betle are suitable for induction of morphogenesis in culture. Tue shoot tip explants were cultured on different concentrations of BAP,IAA and 2,4-D induced profuse growth (Fig: 10-16). Callus, multiple shoots and roots are formed

on medium containing BAP 0.5mg/l +IAA 1mg/l (Fig 15). Frequency was 90,92 and 94% respectively. Among the two explants (leaf base and shoot tip) shoot tip showed best response.

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