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IN VITRO SHOOT INDUCTION IN THE IMPORTANT MEDICINAL PLANT, CYCLEA PELTATA (LAM.) HOOK. F. THOMS. (MENISPERMACEAE)

Biological Science

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ABSTRACT

The present study was aimed at to conserve the Cyclea peltata species, by in vitro regeneration. C. peltata, in vitro regenerations were made by employing tissue culture technology. Direct organogenesis showed that high number of multiple shoots were produce in the MS medium fortified with BAP and Kn (2+1.5mg/L) and also showed maximum percentage of response (1.45%) with the highest shoot length 7.1 cm/ shoot than the other combinations tried. Similarly, Next to this combination the other two combinations BAP and Kn at different concentrations such as 2+1.0 mg/L and 2+0.5mg/L respectively exhibited the significant response (83.30 and 82.25%). The present study concluded that the traditional usage of this plant, Cyclea peltata was standardized with MS media with the addition of growth regulators may use for conserving which type of highly valuable medicinal species from exploitation in its native habitats.

KEYWORDS

Medicinal plants, Cyclea peltata, in vitro regenerations, MS medium.

INTRODUCTION

According to the World Health Organization (WHO) more than 80% of the world population roles on additional medicine for their primary health care and needs (Sanjappa, 2005; Vijayan *et al.,* 2007). The disease preventive properties and medical plants are by holding different phytochemicals, which are non-nutritive chemicals. Due to this huge medicinal values and rapid exploitation of their community are attached bio diversity loss, pollution, environmental degradation etc. Conversation of such important plants is necessary to meet the demand and protect the wild by adopting *in vitro* techniques. Mass propagation of plant species through in *vitro* culture is one of the best and most successful examples of commercial application plant tissue culture technology *in* tissue culture methods was provides new means of conserving and rapidly propagating the valuable rare and endangered medicinal plants (Thesmas and Shankar 2009; Rahman *et al.,* 2008). Plants are the resource of primary and secondary metabolites namely alkaloids, terpinoids, flavanoids, saponins, coumarins, glycosides, phenolics, carboxylic acids, amino acids, sugars, proteins etc. these phytochemicals have significant biological functions and also which contribute specific characteristic and property of the plant. The therapeutic use of the plant is due to presence of these compounds, Hence the study and characterization of such phytocompounds have great significant in pharmacological, antimicrobial and clinical research.

Therefore, in recent years the demand for herbal medicines and several natural products from a variety of plant species is consistently increasing. The ethnic people of Toda's using this plant to treat the disease like ulcer, stomach ache, jaundice, antiprotozoal and antimalarial. The National Medicinal Plants Board of India identified this plant as medicinal plant in high trade sourced from the tropical forest in spite of common claims their remain a poorly investigated plant. Therefore studies were undertaken to conservations of strategies are need up to day. The major objective of present study is conservation of medicinal plant species, *Cyclea peltata* by *in vitro* propagation techniques of three explants such as leaf, node and intermodal explants.

MATERIALS AND METHODS

Species Description Medicinal Uses

Cyclea peltata is distributed in various parts of India including Sri Lanka and Andaman Nicobar islands. In India, it is mainly recorded in Western Ghats of Maharashtra, Karnataka, Kerala, and Tamil Nadu. It is a climbing shrub to twine.

The plant is used in traditional ayurvedic medicine and the root part of the plant is employed as an important ingredient of 1-linguvachadi Choornam' which is used to treat gastric ulcer, allied stomach ailments and malarial disease.

In Vitro Regeneration

Surface Sterilization

The plant parts were washed with running tap water for 15 min followed by 3-4 rinses in double distilled water. These parts were sterilized with 0.5mg of bavastin (fungicide), and rinsed with double distilled water. Further, for removing bacteria, the explants were sterilized with amphicillin and rinsed with double distilled water. Next, they were washed with alcohol (70%) and followed by 0.1% $HgCl_2$ (mercuric choloride) every time which is rinsed with double distilled water after chemical treatment.

Preparation Of MS Medium And Growth Regulators

Composition of MS medium

Explants were inoculated on MS (Murashige and Skoog, 1962) medium containing 3% of sucrose solidified with 0.8% agar (Tissue culture grade, HiMedia, India).

Growth Regulators And Their Preparations

Three groups of growth regulators *viz.,* Auxins, Cytokinins, were used in the experiments. The required volumes of growth regulators were aided o the MS medium before autoclaving and were prepared in different concentration, all the growth regulators were stored at 4°C for further use.

Auxins And Their Preparations

Four Auxins namely, lndole-3-acetic acid (IAA), indole-3-3butric acid (IBA) and a-Naphthaleneacetic acid (NAA) were used in the experiments. The stock solution was prepared by dissolving 10 mg of Auxin individually in 1 mL of NaOH or absolute alcohol. The volume was made up to 10 mL with sterile distilled water.

Cytokin In And Their Preparation

The stock solution was prepared by dissolving 10mg of 6-benzylamnopurine (BAP) and kinetin [6-(furfuralamino)purine] (Kn) in 1 mL of 0.1N Hydrocholoric acid (HCL) and the volume was made up to 10mL by adding sterile distilled water.

Preparation Of The Medium

Double distilled water was used for preparing the medium. The nutrient medium basically consists of inorganic nutrient such as Carbon sources, irons, vitamins, and amino acids the chemicals were weighted accurately in electronic weighting balance (Shimadzu AY 220). All the stock solutions were prepared and stored in well stoppered sterilize bottle and kept in a refrigerator at 4°C. The stock solution is make up into 950m1 in 1 litter beaker. Then the 950 ml solution was splitted into 45m1 of 20 different concentrations which were taken in beaker. Further, the growth regulators were added with necessary combinations and concentrations. Finally the pH was

adjusted to 5.6-5.8, with either 0.1 N NaOH or 0.1 N HCL, using pH meter (Systronics). In each of the 20 beakers, the stock solution (45m1) was made up to 50 ml using distilled water. 0.4g of agar was added in water bath and the medium was dispensed into 100m1 of boiling test tube (10 ml each and after tubes covered by cotton plug then autoclaved at 151bps for about 15 min at 121°C. The autoclaved medium in the culture tubes were cooled and allowed to solidify and it was stored in dark condition for further use. In all tubes after 4 days to ensure that were free from contamination.

Culture Condition

All the cultures were maintained under white fluorescent light (Philips, India) having 3000 lux light intensity in the incubation temperature was 25±2°C and relative humidity of 65-70 % with 16 hours light and 8 hours dark period in every 24 hours cycle.

RESULTS

***In Vitro* Regeneration Studies**

The shoot induction from leaf, node and internodal explants of the species, *Cyclea peitata* were tried to using various concentrations and combinations of growth regulators such as BAP, IBA, NAA, Kn and IAA in the MS medium Among the three explants, the significant response of shoot induction were noticed in the nodal part of *Cyclea peltata,* Further, the nodal explants were tested for the direct shoot induction on standard MS media with 20 different concentration of growth regulators (Table 1). Interestingly the concentration of growth regulators BAP and Kn (2+1.5mg/L) showed maximum percentage of response (91.45%) with the highest shoot length 7.1 cm/ shoot than the other combinations tried (Plates III (1) a, b and c). Next to this combination the other two combinations such as BAP and Kn at different concentrations such as 2+1.0 mg/L and 2+0.5mg/L respectively exhibited significant response (83.30 and 82.25% respectively). However, the combinations of growth regulators, BAP and IBA, BAP and NAA and BAP and IAA were revealed moderate shoot level of shoot formation (74%) at 1 and 0.5 mg/L, 1.5 and 0.5 mg/L and respectively.

Table 1. Effect Of Different Concentration And Combination Of Growth Regulators For Shoot Induction Using Nodal Explants Of Cycalea Peltata.

Growth regulators (mg/L)					Days Required	Shoot Formation (%)	Shoot length (Cm)
BAP	IBA	NAA	Kn	IAA			
1.0	0.5	0.0	0.0	0.0	7	71.75	0.3
1.5	0.5	0.0	0.0	0..0	9	50.75	0.7
2.0	0.5	0.0	0.0	0.0	12	50.55	0.8
2.5	0.5	0.0	0.0	0.0	11	49.97	0.8
0.5	0.0	0.2	0.0	0.0	6	49.96	0.4
0.7	0.0	0.2	0.0	0.0	5	58.3	1
1.0	0.0	0.2	0.0	0.0	9	16.65	1.2
1.0	0.0	0.5	0.0	0.0	12	54.5	1.4
1.5	0.0	0.5	0.0	0.0	14	74.95	4.1
1.0	0.0	0.0	0.5	0.0	3	45.5	3
1.0	0.0	0.0	1.0	0.0	12	41.62	1.7
1.5	0.0	0.0	0.5	0.0	9	59.12	1.9
2.0	0.0	0.0	0.5	0.0	7	82.25	4.9
2.0	0.0	0.0	1.0	0.0	5	83.3	5.6
2.0	0.0	0.0	1.5	0.0	8	91.45	7.1
1.0	0.0	0.0	0.0	0.5	12	74.95	2.1
1.5	0.0	0.0	0.0	0.5	14	58.3	1.2
2.0	0.0	0.0	0.0	0.5	16	66.6	0.9
2.5	0.0	0.0	0.0	0.5	11	33.3	0.2
3.0	0.0	0.0	0.0	0.5	9	16.65	1.3

DISCUSSION

The family Menispermaceae contains 68 genera with some 440 species which are low lying tropical areas and some species present in temperate region. Most of the plants are in the form of woody climbers. All most all the plant species are traditionally followed by the ethnic tribes and reported in Ayurvedha and Siddha system of medicine. One such important medicinal plant, *Cyclea peltata* is having medicinal property. Due to this having medicinal property, the local communities of Toda people continuously exploiting this species as medicinal importance, such species are conserved by *in vitro* technologies. The best shoot response (91%) noticed in nodal explants of *Cyclea peltata* in the MS media composed with BAP and Kn (2and 1.5 mg/L). It is a common fact that the higher concentration and lower concentration of auxin will enhances the shoot formation (Alderete *et al.,* 2006; Aditi *et al.,* 2009; Kuldeep and Narendra, 2011). The similar reports were documented by several authors and were highlighted the combination of BAP and Kn could possibly to produce shoot formation in different species (Hemant Sharma *et al.,* 2015). From this study, the species, *Cyclea peltata* have considered as a highly medicinal value one which exhibited many bioactive compounds (Bhagya *et al.,* 2013).

The direct organogenesis in *Tinospora cordifolia* from the nodal explants (Gururaj *et al.,* 2007; Hemant *et al.,* 2015) and *Psoralea corylifolia* (Pandey *et al.,* 2013) from the shoot tips were reported when cultured on MS medium fortified with varying concentration of growth regulators (Danya *et al.,* 2012).

The in vitro regeneration of *C. peltata* using nodal explants was reported by Abraham *et al.* (2010) where, the explants were cultured on MS medium supplemented with 3mg/l of BAP and 0.5mg/l of IAA to induce the highest regeneration (Jyothi *et al.,* 2010). However, in the present work, the use of same growth regulators at the same concentration induced only 1-2 axillary shoots and these failed to induce roots. This may be due to various factors like environmental conditions, genotype of the explant, culture conditions, and concentration of endogenous growth regulators (Chao-Lin *et al.,* 2011). Despite, the wide usage on medicinal properties will urges exploitation of this species which can be overcome by propagation of this plant via standardized MS media. Further studies are needed in future for the isolation of active compounds and protection of wilds through regeneration techniques.

CONCLUSION

Plants have been an important source of medicine for thousands of years. Medicines in common use, such as aspirin and digitalis, are derived from plants, and new transgenic varieties could be created as efficient green production lines for other pharmaceuticals as well as vaccines and anticancer drugs. Tissue culture is useful for multiplying and conserving the species, which are difficult to regenerate by conventional methods and save them from extinction. In conclusion, our present investigation shows that micropropagation of *Cyclea peltata* through *in vitro* is a reliable method for the rapid multiplication of this species. In the current study *Cyclea peltata* has been cultured *in vitro* subculture cycles of multiplication. This protocol will be helpful for rapid and large scale propagation of *Cyclea peltata* through *in vitro* regeneration.

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