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Research Article

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In Vitro Propagation of Clitoria Ternatea L., - A Valuable Medicinal Plant

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Abstract

Clitoria ternatea L. is commonly known as 'butterfly pea' or "shankhapushpi" and it is a traditional ayurveda medicinal plant belonging to the family Fabaceae. The ayurveda drug 'Shankhapushpi' is made from the leaves, roots and seeds of Clitoria ternatea and treated as a worthy tonic for nerve disorders. In vitro propagation is an alternative method for proliferation as well as conservation of the plant. The explants are cultured on MS medium containing different concentration and combinations of growth regulators. Multiple shoot buds were regenerated successfully from the nodal explants and efficiently rooted on half strength MS medium supplemented with IBA. The regenerated plantlets were successfully transferred to the glasshouse, acclimatized and transferred to the field.

Keywords: Clitoria Ternatea L., In Vitro Propagation, MS Medium, Nodal Explants

Introduction

Clitoria ternatea L. is an eye-catching perennial climber with conspicuous blue or white flowers. It belongs to the family Fabaceae and commonly known as "butterfly pea" or "shankhapushpi". The plant is native to south-east tropical Asia and distributed in India, Philippines and Madagascar [1]. It has a great traditional therapeutic value to treat various complaints [2, 3]. Roots, seeds and leaves are commonly used in the ayurvedic system of medicine and extracts of this plant used as an ingredient for 'Medhya Rasayana' as a rejuvenating recipe used for treatment of neurological disorders and considered as an enhancer for intelligence [4]. The whole plant and seed extracts are used for stomatitis, piles, sterility in females, hematemesis, insomnia, epilepsy, psychosis, leucorrhea and polyuria [5]. The roots are bitter, refrigerant, laxative, intellect-promoting, diuretic, anthelmintic, tonic and are useful in dementia, hemicranias, burning sensations, leprosy, inflammation, leucoderma, bronchitis, asthma, pulmonary tuberculosis, ascites, fever, otalgia, hepatopathy and as a cathartic [6]. The root, stem and flower are also used for the treatment of snake bite and scorpion sting [7]. *C. ternatea* has number of pharmacological activities such as possessing anxiolytic, antidepressant, anticonvulsant, anti-stress [8], sedative [9], antipyretic, anti-inflammatory, analgesic anthelmintic and anti-microbial activities. The extract of *C. ternatea* used to improve acetylcholine content, acetyl cholinesterase activity in rats, learning ability, enhance memory also increase apical and basal dendritic branches [8-14].

The present study of *Clitoria ternatea* has been under taken due to its pharmaceutical significance and the wild stock is quickly diminishing due to over exploitation. Zero effort has been employed for the replacement of such important wild plants that's why this species identified as rare plant species by the International Union for Conservation of Nature and Natural Resources (IUCNNR). The propagation of plant through seed results in less survivability under natural conditions [15]. Therefore, to fulfill the increasing demand of this potent medicinal plant, *in vitro* culture and micro propagation could be an alternative method to assist its conservation.

Materials and Methods

Clitoria ternatea L. plants were collected from Botanical Garden at Osmania University, Hyderabad. *Clitoria ternatea* L. is a climbing slender plant with twining woody stem and opposite petiolate leaves, entire, smooth shiny, varying in shape and size according to their age. Flowers are small, in axillary sessile racemes. The root is long, rigid and cylindrical. These plants were subjected to tissue culture and a good protocol for micro propagation was developed to aid in its multiplication and conservation.

The micro propagation studies comprised the in vitro culture of

nodal explants of *Clitoria ternatea* L. on defined culture media under standard growth conditions. The nodal explants were collected from mature and healthy field grown plants. They were washed under running tap water for 20 min followed by soaking in 0.1 % (v/v) liquid detergent Tween-20 for 5 min and then subsequently washed with tap water. The explants were then soaked in 70% ethanol for 5 min followed by washing with water. Finally the explants were surface sterilized with 0.1% solution of mercuric chloride for 3 to 5 min followed by thorough rinsing in sterile distilled water. A total of thirty explants were inoculated in culture tubes containing MS medium augmented with 2 % sucrose and 0.8 % agar and different combinations and concentrations of various plant growth regulators. The experiment was carried out in triplicates. Prior to that, the pH of the medium was adjusted to 5.8, autoclaved at 121° C for 15 lbs / cm^2 for 15 min and allowed to cool before inoculation. The culture media comprised of the following: MS + BAP (0.5, 1.0, 1.5 and 2.0 mg/l) and MS + BAP (0.5, 1.0, 1.5 and 2.0 mg/l) + IAA (0.5 mg/l). All the inoculated cultures were incubated in sterile growth room under controlled conditions of 22 ± 1° C temperature, 75 % humidity and 2000 lux illumination of 16 hr / 8 hr L/D cycle. The 2 cm long regenerated shoots were transferred to root inducing media comprising half MS medium supplemented with IBA (0.5, 1.0 and 1.5 mg/l).

The regenerated plantlets were later transplanted to pots containing a mixture of soil and vermicompost in the ratio of 2:1. The plantlets were gradually acclimatized on the laboratory bench by covering with a plastic bag with holes (to maintain high humidity), which were opened up gradually over a period of one week. The plants in the pots were moved to the glasshouse to a shaded area and gradually acclimatized.

Results and Discussion

The blue flower variety of *Clitoria ternatea* L. (fig. 1A) was taken up for the study comprises *in vitro* culture studies to develop a good protocol for micro propagation. The nodal explants of Clitoria ternatea L. were cultured on full strength MS medium using different combinations and concentration of growth regulators. An efficient micro propagation protocol was developed with a high percentage of shoot regeneration (fig.1B) and multiple shoots (fig.1C). The highest response of 4.80±1.81 for production of multiple shoots was recorded with MS + BAP (2.0mg/l) followed by 4.23±2.01 in MS + BAP (1.5 mg/l) (Table-1). The explants proliferated by 5-8 days and shoot regeneration was observed by 10-15 days (fig.1D). Shoots of about 2 cm with 2-3 nodes were produced by 25 days. These were cultured on root induction media containing IBA (0.5, 1.0, 1.5 mg/l) to induce roots. The higher concentration of IBA (1.5 mg/l) produced better rooting efficiency of 12.93±0.116 (Fig.1E; Table - 2). The regenerated plants were transferred to the glasshouse for acclimatization (fig.1F). Out of a total of 720 explants (pooled from triplicates) inoculated, 476 explants could regenerate shoots and 190 shoots were inoculated on rooting media for root induction out of which 152 shoots could develop roots to enable 121 plants to be transplanted out of which 96 plants survived in pots.

In the present study, two combinations of growth regulators were used (BAP + IAA) and BAP alone whereas, reported the regeneration of *Clitoria ternatea* L. through the combinations of BAP, KN individually and IAA combined [16]. The present report agrees well with the above report with supplementation of BAP and IAA but the higher frequency of regeneration was obtained with BAP (2.0 mg/l) alone in the present report [17]. reported the plant regeneration of *Clitoria ternatea* L from leaf explants on Driver and Kuniyuki medium supplemented with BAP (2.0 mg/l) and NAA (1.0 mg/l) whereas, in our present report the BAP alone produced the highest shoot regeneration frequency without any additional supplementation of NAA. In the present study it was observed that MS + IBA combination produced efficient rooting compared to above reports where they achieved rooting on MS medium supplemented with NAA. This efficient high frequency plant regeneration protocol developed presently can be taken up for large scale micro propagation for its multiplication and conservation.

Table : Effect of Culture Media on Shoot Regeneration and Production of Multiple Shoots from Nodal Explants of *Clitoria Ternatea* L.

Culture medium	No. of explants with shoot induction	Percentage of shoot induction* (Mean±S.E)
MS + BAP (0.5 mg/l)	56	3.14±1.00
MS + BAP (1.0 mg/l)	60	3.58±1.79
MS + BAP (1.5 mg/l)	68	4.23±2.01
MS + BAP (2.0 mg/l)	77	4.80±1.81
MS + BAP (0.5 mg/l) + IAA (0.5 mg/l)	51	1.55±0.22
MS + BAP (1.0 mg/l) + IAA (0.5 mg/l)	52	1.76±0.45
MS + BAP (1.5 mg/l) + IAA (0.5 mg/l)	54	2.00±0.89
MS + BAP (2.0 mg/l) + IAA (0.5 mg/l)	58	2.15±0.92

*The value was calculated as the percentage of nodal explants that have produced shoots out of the total number of inoculated explants (90).

Table 2: Percentage of Root Induction from Multiple Shoots of Nodal Explants of *Clitoria Ternatea* L

Culture medium	No. of shoots with root induction	Percentage of root induction* (Mean±S.E)
MS + IBA (0.5 mg/l)	50	8.82±0.043
MS + IBA (1.0 mg/l)	60	10.00±0.124
MS + IBA (1.5 mg/l)	80	12.93±0.116

*The value was calculated as the percentage of shoots with root induction out of the total number of inoculated shoots.

Figure 1 (A-F): A. *Clitoria ternatea* L. plant, **B**. Shoot regeneration from nodal explants, 12 days after inoculation, **C**. Multiple shoots, 25 days after inoculation, **D**. Rooting from regenerated shoot, 15 days after inoculation of shoot, **E**. Acclimatization of regenerated plantlet, **F**. Regenerated plant transferred to the field.

The present study describes the successful development of rapid micro propagation protocol of *Clitoria ternatea* L. that can help in the conservation of the valuable medicinal plant which is used in treating various disorders. The protocol developed presently can be taken up in large scale to produce the planting material for development of medicinal plant cultivation programs and it can also help the pharmaceutical industry.

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