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IN VITRO MICROPROPAGATION OF PSORALEA *CORYLIFOLIA*: AN ENDANGERED MEDICINAL HERB

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ABSTRACT

Psoralea *corylifolia*(Babchi) is an endangered medicinal herb with significant pharmacological properties. Due to overexploitation and habitat loss, the natural populations of this species are dwindling rapidly. In vitro micropropagation offers a promising approach for the conservation and sustainable utilization of this valuable plant. This research paper reviews the current methodologies and advancements in in vitro micropropagation techniques for Psoralea*corylifolia*, emphasizing its conservation and medicinal applications. Various aspects, including explant selection, sterilization procedures, culture media composition, growth regulators, and acclimatization protocols, are discussed. Moreover, the paper highlights the pharmacological importance of Psoralea *corylifolia*and the potential benefits of in vitro propagation in ensuring its continued availability for medicinal purposes. Future directions for research and practical implications are also considered.

Keywords: Psoralea *corylifolia*, in vitro micropropagation, conservation, medicinal herb, endangered species

INTRODUCTION

Psoralea corylifolia, commonly known as Babchi, is an endangered medicinal herb belonging to the Fabaceae family (1). Native to the Indian subcontinent and other parts of Asia, this plant has been revered for centuries in traditional medicine systems such as Ayurveda, Siddha, and Traditional Chinese Medicine (TCM)The plant holds significant pharmacological importance due to its diverse array of bioactive compounds and therapeutic properties.In recent years, however, Psoralea corylifolia has faced escalating threats primarily stemming from habitat destruction, overharvesting, and unsustainable harvesting practices (2). As a result, wild populations of this valuable herb have dwindled, leading to its classification as an endangered species in many regions.Given its precarious conservation status and medicinal significance, there is an urgent need for conservation efforts and sustainable utilization strategies for Psoralea corylifolia. In this context, in vitro micropropagation emerges as a promising approach to address these challenges In vitro micropropagation involves the cultivation of plant tissues under controlled conditions, typically in a nutrient-rich medium supplemented with growth regulators (3). This technique allows for the rapid multiplication of plants from a small amount of tissue,

offering several advantages over conventional propagation methods.The aim of this paper is to explore the current state of research on in vitro micropropagation of Psoralea corylifolia, with a focus on its conservation and medicinal applications. By examining the methodologies, advancements, and challenges associated with in vitro propagation of this endangered herb, we aim to provide insights into its sustainable cultivation and utilization.Through a comprehensive review of the literature, we will discuss the selection of suitable explants, optimization of culture media composition, manipulation of growth regulators, and techniques for acclimatizing micropropagated plants to ex vitro conditions. Furthermore, we will delve into the pharmacological properties of Psoralea corylifolia, elucidating its traditional uses and therapeutic potential.

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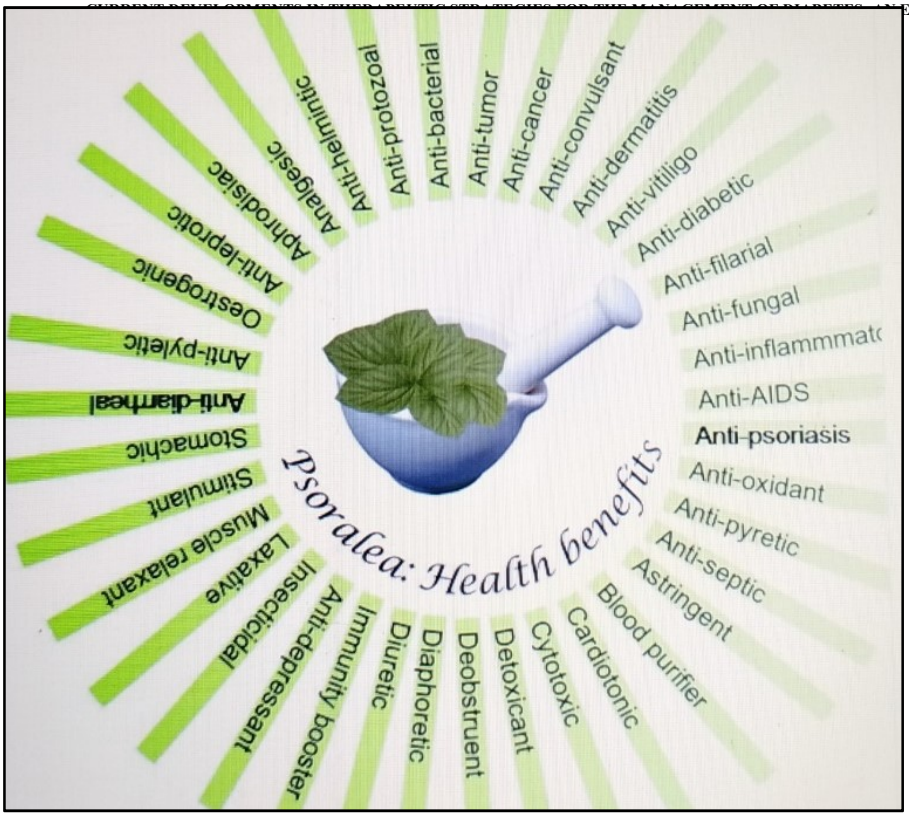

Figure 2: Medicinal properties of Psoraleacorylifolia.

BACKGROUND

Psoralea corylifolia, commonly known as Babchi or Bakuchi, is a perennial herbaceous plant native to the Indian subcontinent and other parts of Asia. It belongs to the Fabaceae family and is characterized by its small, aromatic seeds and trifoliate leaves. Psoralea corylifolia has a long history of use in traditional medicine systems, including Ayurveda, Siddha, and Traditional Chinese Medicine (TCM).In traditional medicine, various parts of the plant, including the seeds, leaves, and roots, have been used for their medicinal properties. Psoralea corylifolia is renowned for its versatile pharmacological activities, including anti-inflammatory, antioxidant, antimicrobial, immunomodulatory, and anti-tumor effects (4). The bioactive compounds present in Psoralea corylifolia, such as psoralen, isopsoralen, bakuchiol, and bavachinin, have been extensively studied for their therapeutic potential.Despite its medicinal importance, Psoralea corylifolia faces numerous threats to its survival in the wild. Habitat destruction, overharvesting for medicinal use, indiscriminate collection, and habitat degradation are among the primary factors contributing to the decline of wild populations. As a result, Psoralea corylifolia has been listed as an endangered species in many regions, necessitating urgent conservation measures to safeguard its genetic diversity and ensure its continued existence.In this context, the development of sustainable cultivation methods for Psoralea corylifolia has become imperative. In vitro micropropagation, or tissue culture, offers a promising solution to address the conservation and cultivation challenges associated with this endangered medicinal herb (5). In vitro micropropagation involves the aseptic culture of plant cells, tissues, or organs in a nutrient-rich medium under controlled environmental conditions. This technique enables the rapid

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multiplication of plant material and the production of genetically identical clones from a small starting population. The application of in vitro micropropagation in the conservation of endangered plant species has gained widespread attention in recent years (6). By providing a controlled environment free from pathogens and environmental stressors, tissue culture offers a means to propagate rare and endangered plants ex situ, away from their natural habitats. In addition to conservation efforts, in vitro micropropagation also holds promise for the commercial production of high-quality plant material for medicinal, agricultural, and horticultural purposes.

MATERIALS AND METHODS

Plant Material:

Healthy and disease-free plant material of Psoralea corylifolia, including nodal segments or axillary buds, was collected from natural populations or maintained in a botanical garden.

Surface Sterilization:

The collected plant material was thoroughly washed under running tap water to remove dust and debris. Subsequently, it was immersed in 70% ethanol for 1-2 minutes for surface sterilization, followed by rinsing with sterile distilled water. The sterilization process was completed by treating the plant material with a suitable disinfectant solution (e.g., 0.1% mercuric chloride or 1-2% sodium hypochlorite) for 10-15 minutes. Finally, the explants were rinsed multiple times with sterile distilled water to remove traces of disinfectant.

Culture Medium Preparation:

Aseptic culture media, including Murashige and Skoog (MS) medium supplemented with various concentrations of plant growth regulators, such as cytokinin's (e.g., benzyladenine, kinetin) and auxins (e.g., indole-3-acetic acid, indole-3-butyric acid), were prepared using autoclaved distilled water. The pH of the media was adjusted to around 5.8-6.0 before autoclaving.

Preparation of Culture media:

The stepwise protocol for preparation of 1 liter MS medium using concentrated stock solutions. Plant growth hormones as per the requirements of the study were added after the MS basal media attains room temperature. pH of the media was adjusted to 5.80-5.88 with 1.0N NaOH prior to autoclaving. Then sucrose 3 %was added as a carbon source and dissolved properly. Finally agar 0.8% was added to the media and volume made up to 1-litre using double distilled water. The contents were boiled to dissolve the gelling agent uniformily.20ml was dispensed in the clean and dry test tubes ,the tubes were wrapped and autoclaved at 121C temperature. The tubes were removed from the autoclave after the steam is completely released and slants were prepared in the slant racks and stored in dust free area until further use.

Table 1: Stock Solutions for MS basal Medium

S.no	Constituents	Amount(gm./lit) (20x)

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1.	**MS I Macronutrients (20x)** i. $MgSO_4.7H_2O$ ii. NH_4NO_3 iii. $Cacl_2.2H_2O$ iv. KNO_3 v. KH_2PO_4	 7.4 33 8.8 38 34
2.	**MSII Micronutrients** i. KI ii. H_3BO_3 iii. $MnSO_4$ iv. $ZnSO_4$ v. $Na_2MoO_4.2H_2O$ vi. $CuSO_4.5H_2O$ vii. $Cocl_2.6H_2O$	 0.166 1.24 4.46 1.72 0.05 0.005 0.005
3.	**MS III Iron salts(100x)** i. $FeSO_4.7H_2O$ ii. $Na_2EDTA.2H_2O$	 2.78 3.73
4.	**MS IV Organic supplements** i. Myo-ionositol ii. Thiamine-HCL iii. Pyridoxine-HCL iv. Nicotinic Acid v. Glycine	 10 0.01 0.05 0.05 0.2

Inoculation: All the aseptic manipulations and inoculations operations were carried out in the laminar flow hood. The working surface was cleaned using 70% ethanol. The door of the laminar flow bench was closed and the working area was exposed to ultra violet light for 30 minutes before work. The plant material was not kept in the cabinet during UV irradiation. The hand and arm were washed with soap and water and then swabbed with 70%ethanol before carrying out the inoculation inside the cabinet. The surgical instruments were cleaned and wrapped in brown paper / aluminium foil and autoclaved. During the course of explant preparation, surface sterilization, inoculations and other aseptic manipulations, all the surgical instruments like forceps, scalpel, needles and blades were dipped into the 70% alcohol and flamed before every operation. During inoculation, first the cotton plug of culture tubes are removed and the neck of the test tube was flamed over the spirit lamp kept in the cabinet. The sterile explants were quickly transferred the test tube containing suitable culture medium using sterilized forceps. The neck of the test tube was again flamed and quickly capped.

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Culture condition: All the inoculated cultures of *P. corylifolia* were incubated in the culture racks present in the dust free culture rooms. The temperature was maintained at 27± 2°C. The cultures were kept in light for 16 h and 8 h dark respectively.

RESULTS AND DISCUSSIONS

Micropropagation is a means of producing large number of plants from a cell, tissue or organ. The micropropagation involves, multiplication of a specific explant, direct somatic embryogenesis, or indirect somatic embryogenesis as an outcome of different combinations and concentrations of Plant Growth Regulators (PGRs). In this study it was found that callogenesis occours at equal concentrations of auxins and cytokinins. When leaf explants were grown at 2.5µM BA and NAA each, callusing was observed. Callusing was also observed to get initiated at 2.5µM BA and IBA each, however the callus died after two weeks. The rate of callus growth was best observed at 3.5 µM BA and NAA each.

Plantlet regeneration via organogenesis was achieved in callus cultures derived from mature leaves of Psoralea corylifolia on Murashige and Skoog medium supplemented with 2.5–3.0 mg L1 BA, 1.0 mg L1 NAA and 3% (w/v) sucrose. The rate of shoot bud regeneration was positively correlated with the concentration of hormones in the nutrient medium.

The basal media developed by Murashige and Skoog's (1962) was used with addition of other concentrations of plant growth hormones given in

Table 2

T.NO	PGR(µM)			Callusing
1	0.00	0.00	-	-
2	0.50	0.50	-	-
3	1.00	1.00	-	++
4	2.5	2.5	-	+++
5	5.0	5.0	-	+
6	7.5	7.5	-	++
7	10.0	10.0	-	-
8	0.50	-	0.50	-

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9	1.00	-	1.00	+
10	2.5	-	2.5	+++
11	5.0	-	5.0	++
12	7.5	-	7.5	-
13	10.0	-	10.0	-

-, +, ++, +++ represent no, slight, moderate and intense callusing

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Figure 3: Callus initiation from leaf explants

The present study described an in vitro micropropagation protocol for Psoralea corylifolia using leaf explant. The disinfected explant were used with MS medium supplemented with BA, NAA, IBA and Kn growth regulator. Media lacking growth regulators was used as control. As early as in 1902, Haberlandt pointed out that excised leaf segments are used as a source of explant material to establish totipotent nature of the plant cells and it has more regenerative potency as any other explants.

The cell differentiation is one central characteristic of plant cells. Plants generate unorganized cell masses, such as callus or tumors, in response to very low levels of PGRs. Callus formation in debarked trees was described over 200 years ago (7). The term "callus" originates from the Latin word *callum*, which means hard. In the early days of plant biology referred to the massive growth of cells and accumulation of callus associated with wounding. Today the same word is used more broadly, and disorganized cell masses are collectively called callus. Callus can be produced from a single differentiated cell, and many callus cells are totipotent, being able to regenerate the whole plant body. Under certain conditions, callus cells also undergo somatic embryogenesis, a process in which embryos are generated from adult somatic cells (8). Thus, at least some forms of callus formation are thought to involve cell dedifferentiation. However, it has also been acknowledged that calli are very diverse and can be classified into subgroups based on their macroscopic characteristics. For example, calli with no apparent organ regeneration typically are called friable or compact callus. Other calli that display some degrees of organ regeneration are called rooty, shooty, or embryonic callus, depending on the organs they generate (9, 10).Exogenous application of auxin and cytokinin in medium induces callus in various plant species. However an intermediate ratio of auxin and cytokinin promotes callus induction, while a high ratio of auxin-to-cytokinin or cytokinin-to-auxin induces root and shoot regeneration, (11). Since the discovery of this regeneration system, it has been widely used, for example, in the propagation of economically important traits and the introduction of transgenes. Other hormones, such as brassinosteroids or abscisic acid, also induce callus and in some species may substitute

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auxin or cytokinin in callus formation (12). However, auxin and cytokinin have been by far the most extensively used and studied hormones in the context of callus formation and subsequent organ regeneration.

CONCLUSION

Psoralea corylifolia is an important medicinal plant species, used in the treatment of various diseases. According to IUCN criteria *Psoraleacorylifolia* has been categorized as critically endangered. The gradual decline in the population of *P. corylifolia*demand launching of conservation effort so as to ensure continuous and ample supply by establishing a balanced cycle of harvest and renewal. Such conservation efforts would ensure continuous and ample supply of this valuable material which is in great demand by the pharmaceutical industry.

The plant is propagated by seed germination. A conventional method of propagation of *P. corylifolia* through seed is not an adequate solution to meet the demand of pharmaceutical industry. To commercialize this plant and to meet the demand for planting material, tissue and organ culture technique are being used as alternative method for propagation in many country.New challenges for refinements of protocols for high rate of shoot multiplication and development of cost effective methods has gained importance in the recent past.

In the present study, protocol was set up for ***Psoraleacorylifolia*** through leaf explant. From the review of the literature, we conclude that in vitro micropropagation can ensure availability of uniform and disease-free propagules for cultivation and management of threatened plants on farmland as conventional breeding is slow and horticultural tools may not be sufficient to meet the future demand for these plants.

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