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Biotechnological Interventions for Sustainable *Ex Situ* Conservation of Medicinal Plants

Dr. Vibha Pandey

Department of Botany
Y.S.N.M. College, Nilamber-Pitamber University

Abstract:
Medicinal plants is experiencing rapid decline due to overharvesting, habitat destruction and climate change, with roughly 40% of the plant species facing extinction. In such a situation, *ex-situ* conservation becomes essential, where species are protected outside their natural habitat. Biotechnology, plays a crucial role in strengthening *ex situ* conservation efforts. Techniques such as tissue culture, cloning, cryopreservation and genetic engineering help preserve genetic material, multiply endangered species and maintain genetic diversity. Through biotechnology, rare medicinal plants can be grown in laboratories in large numbers without disturbing wild populations, ensuring their availability for future use.

Key words: Medicinal plants, biodiversity, *ex situ* conservation, biotechnology, micropropagation.

1. Introduction

Global warming and climate change is posing serious threat to biodiversity. Many species are lost and many others are on the verge of extinction. The ever -increasing human population demand and anthropogenic activities have further exemplified the species extinction rate severalfold [1]. India has 8% of global biodiversity and is one of the 12 mega diversity countries of the world [2]. It has an enormous wealth of medicinal plants which accounts for around 50% of the flowering taxa. Medicinal plants have been used in traditional medicinal systems since ancient times. Use of herbal medicine in cure of various diseases have been described in numerous ancient texts including vedas. The rural and tribal population especially have shown great faith in traditional medicine over several generations. The importance of Medicinal plants lies in its rich and valuable source of industrially important natural plant derived compounds called secondary metabolites which include many terpenes, polyphenols, cardenolides, steroids, alkaloids and glycosides [3]. These secondary metabolites are of immense use in pharmaceuticals, biopesticides, aromatics and food additives. Plants possess innate quality of synthesizing these metabolites naturally in their cells. These compounds are produced in very small quantities and are characterized by extreme complexity in their structure, which often includes multiple chiral centres, large macrocyclic rings and unique linkages. Hence, the chemical synthesis of many of these metabolites is a tough venture [4]. The natural production of secondary metabolites in medicinal plants is nature's profound gift, endowing the plant with survival mechanism on one hand and beneficial to human health on the other. The immense potential of medicinal plants has resulted in its over-exploitation. Further, due to deforestation, degradation and destruction of habitats, the medicinal plant resources are getting extinct [5]. Medicinal plants are also facing regeneration failure in nature. The cumulative effect of the aforesaid factors has resulted in depletion of the existing flora, as a result of which they have been placed in the 'Red list of Threatened

Species' by IUCN [6]. The scientists therefore, found imperative to explore alternate strategies for conservation and mass propagation of medicinal plants.

The advent of Biotechnology provides advanced *ex situ* tools to supplement conventional conservation techniques and facilitate the rapid propagation of medicinal plants. The totipotency of plant cells enables it to develop into whole plants *via* embryo formation. Micropropagation or Tissue culture technology has opened extensive areas of research for mass multiplication and biodiversity conservation [7,8,9,10]. Micropropagation helps in *ex situ* conservation by rapid multiplication of rare species, germplasm conservation, production of secondary metabolites, producing disease free stock and maintaining clonal uniformity.

Tissue culture protocols for a wide range of medicinal plants have been developed and it includes endangered, rare and threatened plant species [11, 12, 13].

2. Important medicinal plants conserved via Micropropagation

2.1 *Rauwolfia serpentina* L.

Rauwolfia Serpentina L., commonly known as sarpgandha or Indian Snakeroot is a woody perennial medicinal herb of family Apocyanaceae. The genus includes around 50 *spp*. distributed widely in the tropical and sub-tropical regions of the world. The plant is of immense medicinal value and the roots of the plant have been used in Ayurveda since ancient times [14,15]. The plant is highly effective against Hypertension [16] and has also been studied for the treatment of mental diseases like schizophrenia, bipolar disorder, seizures, insomnia, sleep related and anxiety problems [17, 18] besides other psycho related disorders.

Rauwolfia contains a wide range of phytochemicals including alcohols, sugars and glycosides, fatty acids, flavonoids, phytosterols, oleoresins, steroids, tannins and alkaloids [19]. **Reserpine** is one of the major alkaloid found in this plant [20]

Rauwolfia Serpentina reproduces through seeds, however, low percentage of seed germination, poor seed viability and delayed rooting limit its natural propagation [20]. Owing to the immense medicinal value of the plant, scientists across the globe prioritised its conservation and developed micropropagation protocols of this important genus. Ahmad *et al*. (2002) [21] used shoot tips from 3-4 year old *Rauwolfia* plant and grew them aseptically for 5-6 weeks. Nodal and leaf explants from such aseptically grown shoot tips were cultured on MS medium supplemented with different concentrations of cytokinins and auxins. It was evident that 0.5 mg/L BA+ 0.2 ml/L NAA was most suitable for callus induction and shoot bud formation. The shoot buds when transferred to 2.0 ml/L BA with comparatively very low concentration of auxin (0.05mg/L NAA), showed excellent elongation of shoot buds. Rooting was induced in excised shoots on half strength MS medium supplemented with varied concentrations of NAA and IBA. Rooted plantlets were kept in a room of normal temperature (30±2°C) at normal daylight for 7 days and were further acclimatized in a potting mixture of garden soil, compost and sand in 2:2:1 ratio. The *in vitro* grown plants were further transplanted in pots containing compost and soil in the ratio 1:1 and it was observed that 95% of the plantlets survived. Similarly, micropropagation protocol has been developed in MS medium supplemented with cytokinins and auxins (BAP and NAA &2,4-D), where BAP (2.0 mg/l) + NAA (0.5

mg/l) has been reported as the most suitable combination for the multiple shoot induction (25.4 shoots per culture). Study has been focussed on large number of multiple shoot production and root biomass production so as to obtain medicinally important alkaloids as well as conserve the natural reserves of *Rauwolfia Serpentina* [15, 22, 23].

Production of Reserpine and other terpenoid alkaloids have been explored in cell suspension cultures. Hairy root cultures induced by *Agrobacterium rhizogenes* have been investigated extensively for the production of terpenoid indole alkaloids. Various biotechnological developments, such as scaling up in bioreactors, pathway engineering etc. have been explored to improve their metabolite production potential [24].

2.2 *Gloriosa superba* L.

***Gloriosa superba* L.**, commonly known as Flame Lily, is an important medicinal plant of family Liliaceae. It is native of tropical Africa and is now growing in many parts of tropical Asia: India, Burma, Malaysia & Sri Lanka. The plant has been used in traditional medicine since ancient times. The plant is semi- woody herbaceous branched climber reaching approximately 5 metres in height. The whole plant possess antioxidant, antibacterial, antimicrobial and anthelmintic properties. The tuber of the plant is used for the treatment of bruises and sprains [25], chronic ulcers, cancer, impotence [26], nocturnal seminal emissions and leprosy [27] besides having anthelmintic, laxative, alexiteric and of abortifacient nature [28].

The plant owes its medicinal properties to the presence of bioactive compounds- Colchicine and Gloriosine [29]. Besides, they are rich in several other biologically active compounds which could serve as potential source of crude drugs that can be used as a complementary source of traditional medicines. It has been used to commit murder, suicide and to induce abortions due to the presence of Colchicine [30].

Gloriosa is propagated naturally by means of seeds, rhizomes or tubers. However, the dormancy of tuber poses challenge in its regeneration. The germination capacity of seeds is also very low besides the hard seed coat [31]. The plant is in severe demand from pharmacological industries all over the world [32]. Its diverse use in medicine, overexploitation for isolation of colchicine, excessive use of planting material for local purpose and susceptibility towards pests have pushed this taxon in the endangered list of plants. The plant material available in nature is not sufficient to meet the demands of the industries. Hence, Scientists across the world felt an urgent need to develop micropropagation protocols of the genus *Gloriosa*, so that the germplasm is conserved and through mass propagation ready availability of plant material is ensured [33].

Ade & Rai (2011) [31] recorded in vitro multiple shoot formation on MS medium fortified with auxins and cytokinins at various concentrations. Yadav *et al*. (2015) [34] recorded multiple shoot formation on MS medium supplemented with different concentrations of BAP or Kinetin individually or in combination with NAA. *In vitro* regenerated shoots were rooted in half-strength MS medium supplemented with different concentrations of BAP or Kinetin individually or in combination with NAA. In vitro regenerated shoots were rooted in ½ strength MS medium containing various concentrations of auxins- IBA & NAA. Transplantation into soil is possible only after the plants form tubers [35]. Sivakumar *et al*. (2004) [36]worked upon the *in vitro* supply of exogenous precursors for the biosynthesis of Colchicine using B5

medium from *Gloriosa superba* L. calluses. Determination of colchicine in callus samples has been established using high performance liquid chromatography combined with mass spectrometry. The maximum amount of colchicine, i.e. 9.0 mg/g DW was detected in the medium fed with 30 µM tyrosine. Sivakumar and Krishnamurthy, 2004 have also reported enhanced *in vitro* production of Colchicine in *Gloriosa superba* L. Nikhila *et al*. (2017) [37] reported colchicine production from suspension culture of *Gloriosa superba* L. Pandurangan and Philomina (2010) [38] have investigated the effect of nutritional factors and the precursors on colchicine production in callus cultures of G. superba in order to optimize the colchicine production in vitro. They reported highest colchicine content in callus grown in medium with sucrose as carbon source and 40 µM ammonium nitrate as nitrate source showed the greatest promise with highest biomass and colchicine content.

2.3 *Withania somnifera*

Withania somnifera (family: Solanceae), commonly known as Indian ginseng is a perennial, erect shrub with green flowers and red berries. It is a xerophytic plant, found in dries parts of India, Sri Lanka, Afghanistan, Baluchistan and Sind and is distributed in the Mediterranean regions, the Canaries and Cape of Good Hope. It is found in high altitude ascending to 5,500 feet in the Himalayas, commonly in Bombay and Western India, occasionally found in Bengal. It grows wildly throughout India particularly in hotter parts, on waste places.

The medicinal value of plant has been proven since Ayurvedic times. The shrub is a potent adaptogen and aphrodisiac used in impotency, cancer, frequent miscarriages, uterine weakness, infertility, asthma, anaemia, arthritis, anxiety, stress, depression, ADHD, cerebellar ataxia, diabetes, high cholesterol, infertility, Parkinson's disease, fibromyalgia etc. Ashwagandha shows positive effects on the endocrine, cardio and central nervous systems [39, 40, 41].

The plant owes its medicinal value to the presence of major biochemical constituents belonging to a class of compounds called Withanolides [42]. Withanolides are secondary metabolites produced via oxidation of steroids and structurally consist of a steroid backbone bound to a lactone or its derivatives [43]. The two main Withanolides are Withaferin A and Withanone.

Large scale cultivation of high value medicinal plant is creating new dimensions in the field of agriculture. However, limited knowledge of seed biology poses a challenge in its cultivation. A rare medicinal plant, its seed germination is poor and the search of elite specimen for its propagation is still under process. To overcome such challenges, several *in vitro* protocols have been developed in *Withania somnifera* [44, 45, 46, 47, 48]. *In vitro* shoot multiplication have been achieved from shoot tips of aseptically germinated seedlings using low concentration of BA (Benzyl adenine) and IBA (Indole Butyric Acid). Direct multiple shoot initiation from germinating seeds has been observed in the presence of BA alone. Kulkarni *et al*. (2006) [49] have reported direct shoot regeneration from node, internode, hypocotyl and embryo explants of *W. somnifera.* Initiation of multiple shoots have been reported on MS basal medium supplemented with various concentrations of BA in case of all explants. Indirect adventitious shoots regeneration via callus culture has been obtained on MS medium supplemented with BAP and Kinetin. Rooting was obtained on growth regulator free half-strength MS medium. Direct rhizogenesis from in vitro leaves has been obtained

by using an IBA dip treatment. These roots were formed along the midrib region of the abaxial surface when placed on MS basal medium.

Vitali *et al*. (1996) [50] have reported Withanolide synthesis in in vitro culture of *W. somnifera* using HPLC. Wadegaonkar *et al*. (2005) [51] have obtained maximum concentration of Withanolides in the bioreactor.

2.4 *Bacopa monnieri*

Bacopa monnieri L. (Family: Scrophulariaceae), commonly known as 'Brahmi' is a perennial, semi-succulent herb found in wet, damp and marshy areas across the globe. In India, the plant has been significant since ancient times, owing to its longevity and mental function. For 5000 years, the plant has been used to treat epilepsy, insomnia and reduce herbal sedation and anxiety [52]. Formulations of this plant has been used as a reputed drug in the Indian Ayurvedic system of medicine since ancient times for treatment of wide range of mental conditions including anxiety, poor concentration, insomnia, depression, epilepsy and Alzheimer's disease [53]. It energizes the central nervous system, aids the circulatory system thus restoring youth and vitality.

The herb owes its medicinal value majorly to the presence of phytochemical compounds, Bacosides and triterpenoids. Besides, saponins, steroids and alkaloids are also present. Bacoside A, is the major compound responsible for neuropharmacological and the nootropic effect of *Bacopa* [54]. Various saponins (Jujubogenin and Pseudojujupogenin derivatives) have been characterized and isolated from the alcoholic extract of *Bacopa monnieri*, e.g. Bacoside A1, A2, A3 Bacopside I, II, III, Bacopasaponin A, B, C, D, E, F, G and H, Bacopaside XI, Bacopaside XII [55, 56, 57, 58, 59, 60].

Due to its immense medicinal value, the herb has been overexploited resulting in its depletion from natural habitat. The herb is endangered and hence alternate strategies are being explored for its mass multiplication. Scientists across the world have developed micropropagation protocols for mass multiplication and ready availability of germplasm [61, 62, 63]. Ali *et al*. (1999) [61] reported multiple shoots from the nodal explants under MS medium supplemented with plant growth regulators such as NAA and BA and additives like glutamine and Copper Sulphate. An average of 15.52±2.77 shoots/ nodal explant was produced within 4 weeks of culture. Tiwari *et al*. (1998) [64] reported production of 79 shoots per leaf explant, 20 shoots/ node and 26 shoots/ internode explant on MS medium supplemented with growth regulators BA, IAA & NAA at various concentrations.

Ahuja *et al*. (2005) [65] have identified production of Bacoside A3 and A2 from in vitro produced shoots of *Bacopa monnieri* through HPLC and LC-MS analysis. Rahman *et al*. (2002) [66] have reported production of Bacoside from suspension cultures of *Bacopa monnieri* (L.) Pennell. Majumdar *et al*. (2011) [67] and Bansal *et al*. (2014) [68] have transformed *Bacopa monnieri* genetically by wild type strains of *A. rhizogenes* which stimulated production of bacopa saponins in transformed calli and plants. Largia *et al*. (2016) [69] have explored elicited Bacoside A contents from *Ri* transformed plants of *Bacopa monnieri* L.

2.5 *Swertia chirayita*

Swertia chirayita, belonging to the family Gentianaceae is a popular medicinal herb native to temperate Himalayas. It has been used in traditional medicine to treat numerous ailments, e.g., liver disorders, malaria, diabetes etc. The herb's medicinal usage has been well documented in Ayurveda, Unani, Siddha and other traditional system of medicine. This ethnomedicinal herb is characterised by the presence of different chemical constituents such as amarogentin (most bitter compound isolated till date), swerchitin, swertiamarin etc [70]. Besides, it also contain active constituents responsible for its therapeutics such as xanthones, flavonoids, iridoids and secoiridoid glycosides responsible for its therapeutics [71]. Ophelic acid, a yellow bitter compound and two bitter glucosides chiratin is also present in *Swertia* [71, 72, 73, 74]. Amarogentin is known to be anti-diabetic, anticancer and anti-arthritic [75, 76, 77]. Owing to its immense medicinal value, the plant has been overexploited leading to it becoming extinct in nature [78].

Swertia chirayita, is harvested early before the seeds mature, hence mass scale propagation *via* seeds is difficult. Scientists have therefore explored alternative reproducible micropropagation strategies for its *ex-situ* conservation, mass propagation, and ready availability of the germplasm [79, 80, 81]. Wawrosch *et al*. (1999) [79] reported adventitious shoot regeneration from root explants on MS medium supplemented with 3 mMBAP. In another study, Chaudhari *et al*. (2007) [80] reported shoot regeneration on half-strength MS basal medium supplemented with 0.44 µM 6-BAP and 4.65 µM 6- furfurylaminopurine. Maximum of 18 shoots were obtained when medium was again supplemented with 10 mM KNO3 and 75mg/l of casein hydrolysate. Micropropagation of *Swertia chirayta* using 4 weeks old seedling derived nodal explants have been reported by Joshi and Dhawan (2007) [79]. Pant *et al*. (2010) [73] developed an efficient micropropagation protocol using nodal segments as explants. Plant regeneration through indirect organogenesis *via* formation of callus has also been reported [81].

The adventitious shoots obtained above were successfully rooted in half strength MS medium supplemented with NAA/IBA [82]. Rooted plants were hardened and transplanted in soil with 80-90% survival rate [83].

Swertia chirayita may also be conserved through Synthetic seed production, another tool of biotechnology [84]. It can also be conserved through cryopreservation [85].

3. Conclusion

Medicinal plants constitute an invaluable component of global biodiversity and serve as a rich source of therapeutic compounds used in traditional and modern medicine. However, increasing anthropogenic pressures, habitat destruction, climate change, global warming and overexploitation have placed many medicinal plant species under severe threat. In this context, biotechnological interventions have emerged as powerful tools for sustainable ex-situ conservation. Techniques such as micropropagation, tissue culture, cell and suspension cultures, synthetic seed technology, cryopreservation, and genetic transformation not only facilitate rapid multiplication and longterm preservation of endangered species but also support the production of valuable secondary metabolites without exerting pressure on natural populations. The successful application of these technologies in medicinally important spp. such as *Rauwolfia serpentina*, *Gloriosa superba*, *Withania somnifera*, *Bacopa monnieri* and *Swertia chirayita*

demonstrates their immense potential in conserving genetic resources and ensuring a sustainable supply of plant material for pharmaceutical and industrial use.

Nevertheless, biotechnology should complement, rather than replace, *in situ* conservation efforts. An integrated approach combining conservation, sustainable utilization, scientific research and policy support is essential for safeguarding medicinal plant diversity for future generations. Continued advancements in plant biotechnology will play a pivotal role in achieving the dual objectives of biodiversity conservation and sustainable development.

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