MICROPROPAGATION OF MEDICINAL PLANTS

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Micropropagation of Medicinal Plants

(Volume 2)

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PREFACE

Micropropagation of medicinal plants has become vital in providing high yielding elite genotypes for pharmaceutical purposes, as well as in producing high quality plantlets for conservation. Due to excess demand and injudicious harvesting, deforestation, climate change, pollution, urbanization, and natural calamities, many medicinal plants are under threat in their natural habitat. Variations in different biotic and abiotic environmental conditions severely limit the conventional method of propagation. As a result, micropropagation techniques may provide a better alternative and make it possible for the rapid multiplication of medicinal plants. It plays a significant role in increasing the production of disease-free plants, regardless of the season, with the goal of restoring these plants in their natural habitat and conserving them. These plants also serve as another source of raw materials used for commercial purposes, reducing the stress on plants growing in natural habitats. Tissue culture protocols for a wide variety of medicinal plants have been developed over the years. It also allows the modification and regulation of their genetic information with the goal of producing valuable phytoconstituents in greater quantities or with better properties, or both.

The present book gives the protocols for micropropagation of more than 40 species of medicinal plants. This book smartly combines the scientific principles with the state of the art in tissue culture techniques presented by experienced and authors. I wish to express my gratitude to all the authors who contributed to the review chapters and research papers. I thank them for their cooperation and erudition. I hope that this will be a source book for the cultivation and improvement of medicinal plants. I request that readers give their suggestions to improve in future editions.

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CHAPTER 1

Micropropagation Studies on Genus *Cissus* A Review

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Abstract: The genus *Cissus* Linn. belongs to the Family Vitaceae (formerly Ampelidaceae) and comprises about 350 species distributed all over the world, having rich phytochemicals with medicinal as well as commercial value. This genus is a storehouse of large varieties of phytochemicals such as alkaloids, flavonoids, and phytosterols, making this genus pharmaceutically important. Some species contain high quantity of calcium ions in their stem extract, which is possibly responsible for their bone healing activity. *In vitro* propagation of plantlets provides the opportunity to conserve endangered species as well as to use the beneficial species without disturbing their natural habitat. The present review comprises *in vitro* protocols used to conserve the species, exploit and enhance useful metabolites. The whole plant, parts and metabolites isolated from *in vitro* cultures of *Cissus* species may be used further for pharmaceutical purposes.

Keywords: *Cissus*, Conserve, *In vitro* propagation, Phytochemicals, Pharmaceutical.

INTRODUCTION

The genus *Cissus* Linn. belonging to the Family Vitaceae (formerly Ampelidaceae) comprises about 350 species [1, 2]. It has been reported by many researchers that the genus has approximately 135 species in Africa, 85 in Asia, 12 in Australia and 65 in the Neotropics [3]. This genus has cosmopolitan distribution across the globe and is characterized by polypetalous flowers having prominent disk-shaped thalamus below the ovary. and represents the largest of the 14 genera of Vitaceae, primarily distributed in tropical and temperate regions of the world [3]. The *Cissus* group of plants have a variety of bioactive properties and are known for their medicinal uses since ages. Diarrhoea, loose stools,

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coughs, and breast cancer are some common diseases that can be cured by the use of various preparations of *Cissus* species.

PHYTOCHEMISTRY OF THE GENUS

Cissus species are being used in all parts of the world and are implicated in treating various ailments. As reported by several researchers, species of the genus *Cissus* are often used as medicinal plants because they contain vitamins, proteins, carbohydrates, and polyphenols Table **1**.

Table 1. Various phytochemicals reported from different species of genus *Cissus* responsible for their pharmacological properties.

Plant Name	Extract	Chemicals Reported	References
C. quadrangularis	Whole plant	Vitamin C, flavonoids, triterpenoids, stilbene derivatives and several secondary metabolites, *e.g.,* quercetin and kaempferol, quadrangularin A-C, resveratrol, pallidol, perthenocissin piceatannol and phytosterols. Calcium ions, β-sitosterol, d-amyrin, onocer-7-ene-3a, 21beta-diol, d-amyrone and 3,3',4,4'-tetrachloro-1,1'-biphenyl. Anabolic steroidal substances and carotene 7-oxo-onocer-8-ene-3-β 21-α-diol have been reported.	[8-11]
C. vitiginea	Methanolic leaf extract	Diethyl phthalate, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, myristic acid, azelaic acid, 2,6,10-trimethyl 14-ethylene-14-pentadecene, 2-hexadecene, palmitic acid, 2-hydrox--1,3-propanedyl ester, 22-tricosanoic acid, oleic acid, dibutyl ester, L-ascorbyl 2,6-dipalmitate, dibutyl phthalate oleic acid, heptadecanoate, 2-hexadecen-1-ol, 1H-cyclopropan-α-aphthalene, 9,12-octadecadienoic acid (Z,Z)-, nonadecanoic acid, palmitic acid, icosanoic acid, octadecanoic acid, 2,3-dihydroxypropyl ester, stearic acid glycidyl ester, and bis(2-ethylhexyl) phthalate.	[12]
C. pteroclada	Ethanolic extract of the roots with stems	Gallic acid, β-sitosterol, bergenin, 11-O-(4-hydroxy benzoyl) bergenin, 11-O-galloylbergenin and daucosterol	[13]
C. ibuensis	Ethanolic leaf extract	Rutinoside and quercetin.	[14]
C. assamica	Whole plant	Lupeol, β sitosterol, n-hexacosinic acid, daucostenin, isolariciesinol-9-O-beta-D-glucopyranoside, 3,8-Di-O-methylellagic acid and bergenin.	[15]
C. repens	Ethanolic extraction of aerial part	Stilbene C glucosides.	[16]

Good quantity number of Ca^{2+} and P ions, essential for bone growth, have been reported from *Cissus quadrangularis* stem extract [4]. Fracture healing studies on *C. quadrangularis* have also been reported by some Indian laboratories (Bulletin of Department of Pharmacology, Nagpur, 2002). *In vitro* screening and pharmacological studies [5] and chemical components [6] of *C. quadrangularis* have also been reported. *C. quadrangularis* has also been used to synthesize calcite crystals [7].

Advanced biochemical analyses of various species of *Cissus* have revealed the presence of a number of useful phytochemicals summarised in Table **1**.

MICROPROPAGATION

Micropropagation has the upper hand over vegetative propagation methods as it can rapidly multiply valuably genotypes, release improved varieties and disease-free plants at a quick pace. It is also important for off-seasonal production of plantlets, germplasm conservation and secondary metabolites production.

The plant tissue culture technique has been tremendously exploited for *in vitro* propagation of desired genotypes on a mass scale. The results of such studies have practical as well as economical value-producing industrially valuable compounds [17]. This technique is a potential renewable source of obtaining valuable compounds from a particular species, we can also get flavours, fragrances, colourants, which cannot be produced by microbial cells or chemical synthesis [18]. Therefore, plant cell culture is being utilized for extensive production of valuable medicinal plants these days.

Need for *in vitro* Propagation of Genus *Cissus*

All the species belonging to keep it genus *Cissus* are medicinally important as they possess useful antiarthritic, antidiabetic, anticholestrolemic, anticancerous, anticonvulsive, antimicrobial, and anti-inflammatory properties as indicated in Table **2**.

Table 2. The effect of the *Cissus* plant part on target organisms showing various activities.

Plant Name	Useful Plant's Part	Activity	Target Organism	References
Cissus quadrangularis	Stem and root	Bone healing activity,	Murine osteoblastic cell line, Wistar albino rats	[86]
Cissus adnata	Whole plant parts	Antioxidant activity	-	[87]

CHAPTER 2

Micropropagation of *Juglans regia* L.

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Abstract: *Juglans regia* L., commonly called walnut, is a nutrient-rich fruit. Besides many therapeutic properties, the plant is highly valued for its timber, which along with the fruit-nut, fetches a very high demand in the domestic and international markets. The ever increasing demand for these plant products is not being sufficed by the existing supplies. This is owing to the fact that conventional methods of walnut propagation are time and space-consuming and show limited responsiveness. Walnut cuttings are also difficult to root, making large-scale propagation a challenge. Consequently, walnut micropropagation has become extremely important to ensure rapid mass production of selected cultivars in a small space, and for an indefinite time period. The tissue-culture-raised products are robust, disease-free, and have desirable characteristics. The aim of this chapter is to compile information on tissue culture studies on *Juglans regia* with a special focus on the latest developments in the field. The chapter covers various pathways employed for the *in vitro* propagation of walnut, hardening, and acclimatization of tissue culture raised plantlets to ensure better quality, quantity, and sustainability of walnut trees to meet the demand of the growing global population.

Keywords: Callus Culture, Embryo Culture, Hardening, *In vitro* Rooting, *Juglans regia*, Nodal Explant Culture, Somatic Embryos, Walnut.

INTRODUCTION

Juglans regia L. (family Juglandaceae) is one of the most popular fruit-nut trees. Commonly known as Persian walnut, English walnut or walnut, is native to the region that runs eastward from the Balkans to the western Himalayan Mountains [1]. The edible seeds of the drupe on any Juglans tree are known as walnuts. The grain is encircled by the shell. It typically has a fibrous shell barrier that breaks as it ages, dividing it into two halves. The brown seed coat that surrounds the seeds, which are often sold as walnuts without the shell, contains antioxidants. This nut is referred to as the King of Nuts because of its ultimate benefits.

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Walnuts contain significant amounts of fats, proteins, vitamins, and minerals, making them nutrient-dense food and accounting for their extensive usage in the traditional system of medicine. The average amount of protein in walnuts is around 18%, wherein glutelins make up around 70% of the total protein in seeds, and have 18% globulins, 7% albumins, and 5% prolamins [2 - 6]. Potassium, magnesium, and calcium are known to be the most prominent nutrients in walnuts abundance in walnut cultivars [7 - 9]. They are also rich in phenolic acids, related polyphenols, pectic compounds, sterols, flavonoids, and sterols. The nutritional composition varies between cultivars and varies with factors like genotype, cultivator, environment, and soil type [10 - 12].

The phytochemically active ingredients of the species are responsible for its wide range of therapeutic properties like antioxidant, anti-hypertensive, antihistamine, bronchial relaxant, analgesic, neuroprotective, immuno-modulator, antiulcer, antidiabetic, hepatoprotective, antibacterial, anti-inflammatory, lipolytic, dental-cavity preventive, and many others. Walnuts have high amounts of omega-6 and omega-3 polyunsaturated fatty acids, which prevent heart-related ailments [13]. The high protein and oil content of the walnut kernels has led to the FAO (Food And Agriculture Organization) designating the walnut as a priority plant and a key species for human nutrition [14]. Besides, the wood is robust and solid, making it a top choice for high-end furniture and woodwork. Due to its significant commercial value and great health advantages, it is a crop that is in high demand in both domestic and international markets.

Conventional methods of walnut propagation by cutting or grafting are cumbersome and less productive, as is the case with many other woody plant species. One of the cutting-edge and important techniques for plant multiplication is the tissue culture technique, sometimes referred to as micropropagation [15 - 18]. A lot of plants may be produced quickly using the micropropagation method, which is especially advantageous when compared to vegetative propagation or when regeneration species like walnuts are difficult to regenerate naturally.

The present chapter discusses the different methods of mass propagation of *Juglans regia* using different techniques of plant tissue culture.

PLANT TISSUE CULTURE GROWTH MEDIUM FOR WALNUT

One of the most crucial elements of plant cell and tissue culture is the growth media. Different culture media have been employed for the micropropagation of walnut, including Murashige and Skoog medium (MS), Driver and Kuniyuki medium (DKW) and Woody plant medium (WPM) with varying success. The most used media for walnut tissue culture is the DKW medium, which was created for the cultivar paradox and has proven useful for a number of

Juglandaceae species, including *J. regia* [19 - 22]. However, a number of researchers have successfully cultured Persian walnut using MS media [23 - 26]. The suitability of DKW medium for walnut propagation is attributed to the fact that walnut requires high salt media for shoot multiplication, a condition which is provided by DKW medium [27 - 30].

Countering Metabolic Exudates

Growing walnuts has been significantly hampered by the phytotoxic exudates that are produced by recently cultivated explants [31]. Metabolic byproducts frequently cause the culture media underneath the explant to become darker. To regulate these exudes, a number of techniques have been explored. These include soaking the seed explants in water for twelve hours [32], use of antioxidants like cPIBA (cyclo-pentano-isobutyric acid), TIBA (2,3,5 tri-iodobenzoic acid), ascorbic acid, phloroglucinol, sodium thiosulfate, dithiothreitol and charcoal [33, 34]. A study with mature tissues (glabrous shoot tips, internodal segments) of field-grown *J. regia* highlighted the use of fungicide solution (Captan + Benomil 1g/l each) and antioxidant solution (20mg/l cysteine + 5mg/l ascorbic acid) to establish healthy cultures [35].

In general, periodic transfer of explants to new media has proven to be more effective in eliminating the exudate problem. The process is continued till the release of exudates from the cultures subsides.

APPROACHES FOR *IN VITRO* PROPAGATION OF *JUGLANS REGIA*

Nodal Explant Culture

Juglans regia and hybrids are often micropropagated from shoots, branches, zygotic embryos, or adult plant scions [29]. In one of the initial studies, incubation of explants in a modified MS medium supplemented with BAP for 4 weeks resulted in longer axillary buds from cultured explants [36]. A study [23] was carried out to improve walnut micropropagation procedures using embryonic and juvenile nodal explants. MS medium supplemented 1.0 mg/l IBA was most optimal for *in vitro* shoot development. The study showed that higher levels of cytokinin BAP(6-Benzylaminopurine) induced deformation of leaves and shoots, eventually causing vitrification, while GA_3(Gibberellic acid) promoted elongation of shoots. Another study [16] showed that a modified MS medium with BAP proved to be most optimal for shoot induction from immature herbaceous shoots of *Juglans regia.* However, for further development, DKW medium containing IBA,2.0mg/l and riboflavin1.0mg/l proved to be most optimal when cultures were given a ten-day darkness period. Through this system, *in vitro* regenerated plantlets could be transplanted after 4-5 weeks. A study on *in vitro* propagation of

CHAPTER 3

Micropropagation and *in vitro* studies in *Alpinia* Roxb. (Zingiberaceae)

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Abstract: A tropical and subtropical Asian genus called *Alpinia* is used for both horticultural and medicinal purposes. Species having ornamental uses are now distributed widely all over the world. Different species of *Alpinia* are widely used in traditional medicine for treating many diseases. Several *Alpinia* species have now been experimentally demonstrated to have medicinal properties. Excess trade of many species of *Alpinia*, such as *A. calcarata*, *A. galanga etc*., as well as habitat loss and urbanization demands its mass propagation. Therefore, one of the best methods for its mass propagation and conservation is micropropagation. *In vitro* studies of medicinal taxa such as *A. calcarata*, and *A. galanga* and ornamental species such as *A. purpurata* has been well established. Different *in vitro* approaches such as direct organogenesis, callogenesis and indirect organogenesis, somatic embryogenesis (SEs), and multiplication using inflorescence buds were generally tried for the successful micrpropagation of different species of *Alpinia*. Genetic and phytochemical fidelities of the *in vitro* raised plants were also studied in many instances to enhance the commercial use of it.

Keywords: *Alpinia calcarata*, *A. galanga*, *A. purpurata*, *A. zerumbet*, *Alpinia malaccensis*, Callus, Clonal Propagation, Explant, Genetic Fidelity, Hormones, *In vitro* Propagation, Meristemoids, Organogenesis, Plant Growth Regulators, Rhizome Buds, Somatic Embryogenesis.

INTRODUCTION

Alpinia is the largest genus in the family Zingiberaceae, and comprises approximately 245 species worldwide and it is native to Tropical and Subtropical Asia to West Pacific [1]. The species of *Alpinia* have been widely used for various purposes for centuries. *Alpinia* is widely used as traditional medicines in China,

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India, and Japan to treat various diseases such as gastralgia, indigestion, vomiting, *etc*. Research on *Alpinia* species revealed its pharmaceutical and pharmacological properties scientifically, including antianxiety, antibacterial, antiemetic, antifungal, antitumor, antiulcer, cardioprotective, hypoglycemic, and neuroprotection activities. Many species are cultivated for its ornamental uses such as *A. calcarata*, *A. purpurata*, *A. zerumbet*, *etc*. *Alpinia blepharocalyx* is used as a natural dye [2]. The species namely *A. galanga* is a multipurpose plant has variety of uses. It is widely used by the traditional medicinal practitioners due to its ethnomedicinal properties such as anti-inflammatory, antioxidant, antimicrobial, anticancerous, spasmolytic, properties [3 - 5]. The therapeutic uses of *A. galanga* are due to its medicinally active constituents such as α-fenchol, α-fenchyl acetate, myrcene, 1, 8-cineole, camphor, camphene, *etc* [6]. Compounds namely acetoxyeugenol acetate, acetoxychavicol acetate (ACA) and p-coumaryl diacetate characterised from *A. galanga* are well-known for its anti-HIV, anti-parasitic, anti-tumour, and antituberculous activities [7 - 9]. Besides, the rhizome has also been used as condiment in many parts of the world [5, 10]. The pharmacological and pharmaceutical properties of other species of *Alpinia* such as *A. katsumadai*, *A. oxyphylla*, *A. calcarata*, *A. purpurata*, *A. zerumbet* has also been proved recently [2].

Due to deforestation, climate change, natural calamities, indiscriminate harvesting, commercial exploitation, and pollution, plants in natural habitats are in great decline [11]. Consequently, these plants are under threat or become endangered day by day, hence demanding their conservation. Moreover, the conventional method of propagation either could not be accomplished in many plant species or may not be adequate to fulfill the demand of many species, especially medicinal plants. These impediments can be avoided through biotechnological methods of propagation such as *in vitro* propagation [12]. Micropropagation of *Alpinia* species is quite occasional, with only about less than 10 species studied so far. The *in vitro* method that was most extensively studied was direct organogenesis from axillary meristems of rhizomes, though callus induction and somatic embryogenesis were also established in some species. This chapter outlined the different methods used for the micropropagation of the medicinal herb *Alpinia*.

MICROPROPAGATION OF *ALPINIA* SPECIES

Alpinia calcarata Roscoe

Alpinia calcarata is a species that can be found in moderate distributions, primarily originating from South India and extending to China (Guangdong) and Indo-China. This plant is a rhizomatous geophyte and thrives in wet tropical

biomes [1]. With its diverse medicinal properties, clonal propagation of *A. calcarata* has been carried out to some extent.

Clonal Propagation from Axillary Rhizome/Shoot Buds

Sudha and co-workers [13] studied the requirements of optimum concentration and composition of plant growth regulators (PGRs) in clonal propagation of *A. calcarata.* The explant used were axillary shoot buds taken from a 12-month-old rhizome of field-grown plants. After proper disinfection, the explants were cultured on Murashige and Skoog (MS) medium [14] fortified with thidiazuron (TDZ), 6-benzylaminopurine (BAP) and kinetin (Kn) at a concentration of 0.1-5.0 mg/L for the initiation of shoot buds. For the standardization of ideal concentrations of auxins (0.05-5.0 mg/L), such as 1-naphthylacetic acid (NAA), indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA) with 2.0 mg/L BAP were tried. After 8 weeks of initiation, the regenerated axillary shoot buds were subcultured on MS medium augmented with a combination of BAP and 0.2 mg/L IAA or BAP alone (1.0-3.0 mg/L) for multiple shoot induction. Solitary axillary shoot buds with meristemoids and groups of multiple shoot initials were separately sub-cultured on MS medium augmented with the mixture of 0.1 mg/L IAA and BAP or BAP only (0.5-1.0 mg/L) at the next stage of propagation. Meristemoids and groups of three to four multiple shoot initials were sub-cultured on MS medium augmented with 0.1 mg/L BAP for the maintenance of cultures. ½ strength MS (full-strength sucrose and myo-inositol) liquid and solid medium with varied levels of auxins (NAA, IBA, IAA) alone or in combinations were used for the initiation of roots from well-grown shoots (3.0-4.0 cm) of six-week-old cultures. The role of Kn, BAP, and TDZ on the initiation of the axillary bud was evaluated after 8 weeks of culture initiation. Maximum length and number of shoots were obtained in MS medium augmented with 1 mg/L Kn, 2 mg/L BAP, and 3 mg/L TDZ in the first stage of *in vitro* cultures. A mixture of 2.0 mg/L BAP + 0.5 mg/L NAA gave a significantly higher rate of callusing (39%). The mean number of shoots/explants observed was 5.2, whereas the highest mean length of shoots was 4.8 cm in the combination of 2.0 mg/L BAP + 0.1 mg/L IAA. Maximum multiple shoot initials (12.1) and meristemoids (4.0) were produced on MS medium fortified with 2.0 mg/L BAP and 0.2 mg/L IAA (Table **1**). Once the same propagules were transferred to the third subculture on a medium containing 1.0 mg/L BAP alone, the rate of multiplication was further increased to 32 shoots. Within two weeks of culturing, the meristemoids produced an average of 10 shoot initials at each stage. The best PGR combination for the rooting was 0.2 mg/L IAA and IBA with a response rate of 100%, 9.3 roots/shoot and 6.85 cm mean root length. After 7 months, 500 shoots were produced from a single axillary shoot bud explant by employing the above *in vitro* culture method. When root induction and shoot multiplication were carried out in liquid media, a substantial

CHAPTER 4

Micropropagation of Ginger (*Zingiber officinale* Roscoe)

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Abstract: *Zingiber officinale*, belonging to the family of Zingiberaceae, is commonly known as ginger and is commercially grown as a spice and for culinary purposes. It is a potential Ayurvedic herb with many medicinal properties. A small section of the plant's rhizome is widely used for micropropagation. Besides rhizome explants, callus induction, shoot induction, and meristem culture are used to propagate the plant. For the production of ginger's pest-resistant and disease-free planting material, micropropagation is regarded as the best method. Various classes of bioactive entities, such as flavonoids, alkaloids, glycosides, phenols, tannins, terpenoids, steroids, saponins, and oils, have been identified in the plant. Phenolic bioactives such as gingerols and shogaols are primarily responsible for their therapeutic properties. Various pharmacological activities have been investigated in ginger. This review concentrates on different advanced methods for ginger propagation, especially micropropagation.

Keywords: Ginger, Micropropagation, Multiplication, Plant regeneration, Shoot induction, Zingiber officinale, Zingiberaceae.

INTRODUCTION

Zingiber officinale Roscoe is a perennial herb which is believed to be indigenous to India and China and is widely cultivated in tropical regions such as Southeast Asia, the Caribbean, Central and South America, Australia, and Africa [1]. *Z. officinale* is widely used as Ayurvedic and traditional Chinese medicine because of its promising health benefits. It is widely used to treat numerous health disorders, namely fever, bronchitis, sore throat, digestive issues, nausea, vomiting,

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common cold, abdominal pain, and rheumatism [2]. Raw ginger is dried and powdered and used to make tea, which is usually taken to get relief from cold and cough. *Z. officinale* rhizomes are often chewed raw, and they help in reducing abdominal pain. The rhizomes are usually boiled with other herbal ingredients like basil, turmeric, black pepper, and bay leaf to make *kadha* or decoction, which has many health benefits and helps improve body immunity. During the pandemic of COVID-19, the consumption of ginger-based decoction and tea was recommended by health experts to prevent various respiratory disorders such as cold, flu, and cough. Raw ginger rhizome is a home remedy to prevent nausea and constipation. Ginger has stimulant, carminative, and diaphoretic properties; used to flavour food, beer, and other beverages; used in curries as a condiment. Ginger rhizome possesses diuretic, anti-inflammatory, anti-emetic, and sialagogic properties. Rhizome juice is used to prevent migraine, colic, and catarrh. Moreover, it is also believed to provide relief from pain due to menstrual cramps. Ginger oil is used externally to relieve toothache, headache and pain due to swelling and boils; leaves are consumed in Malaysia to reduce stomach pain and rheumatic diseases. Fresh leaf juices are used externally to reduce the symptoms of ague in children. In China and Indonesia, the ginger rhizome paste is used as an antispasmodic and applied externally as an antidote against snake, fish and crab stings.

Ginger is generally propagated through the rhizome. Using rhizome as a starting material affects the yield and supply in the market as the cultivators have to store a high proportion of rhizome for the next growing season. Moreover, the rhizome is easily affected by bacteria and fungi, the newly grown leaves also get infested by pests easily, and all these aspects adversely affect ginger production. This method of cultivation is costly and even needs more human resources; therefore *in vitro* propagation or micropropagation of the crop will be fruitful for producing healthy planting material, which can help achieve the growing demand for ginger. Various types of explants, such as axillary buds from rhizomes, vegetative buds, shoot tips, young buds, root tips, sprouting buds, and rhizome buds, were used for micropropagation of different ginger varieties [3 - 5]. Micropropagation of ginger in a microbial-free controlled condition aids in producing high-frequency multiplication of disease-free clones of ginger.

The pungent smell of ginger is due to the homologous series of phenolic compounds called gingerols. Studies reported the presence of a wide array of phytochemicals in ginger, such as essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, saponins, steroids, terpenoids and tannin. It is rich in geraniol, α-curcumene, (E, E)- α-farnesene, α-zingiberene, and β-sesquiphellandrene [6]. Phytochemicals present in ginger exhibit good antioxidant, antimicrobial, antiserotonergic, antispasmodic, anticonvulsant,

analgesic, anti-inflammatory, antiulcer, larvicidal, and immunomodulatory activities [7, 8]. Ginger extracts are found to have a potential role in treating Alzheimer's disease and improving the function of the nerve cells [9, 10]. It was reported that in the scopolamine-induced memory deficit paradigm, dried ginger extract at three different doses, containing 6-shogaol (11.7%) and 6-gingerol (5.52%), restored cognitive abilities and delay in the time for the passive avoidance of learning exercise in mice [11]. It was investigated that 10-gingerol (30 M) inhibited 50% of the growth of HCT116 cells with abnormalities characteristic of programmed cell death [12].

Clinical studies among 45 randomized 20-60 age group patients with Type-2 diabetes melitus were conducted where the patients had not been given any insulin treatment for three months. They are subjected to oral administration of powdered ginger (3g/day), which exhibited a significant decrease in blood glucose, triglyceride, highly sensitive-C-reactive protein, and malondialdehyde, and an increase in paraoxonase-1 and total antioxidant capacity (TAC); this indicates that ginger has a potential antidiabetic effect by maintaining blood glucose homeostasis and lowering oxidative stress in the body [13]. Furthermore, it was investigated that the antimicrobial activity of ginger in bacteria forms a zone of inhibition; *Citrobacter* spp. (14 mm), *Escherichia coli* (9.5 mm), *Salmonella* spp. (11.1 mm), *Shigella* spp. (12 mm), and *Enterobacter* spp. (0.66 mm) [14].

MICRO PROPAGATION OF GINGER

Micro-propagation is a method of propagating crops that are infertile and lack natural seeds or reproduce through vegetative propagation. Ginger rhizomes are easily affected by fungi (*Pythium* spp. and *Fusarium oxyporium*) and bacteria (*Ralstonia solanacearum*), which cause rot disease. It spreads from infected rhizomes to whole crop fields and leads to a significant loss in the production of ginger [15, 16]. To protect the plant from pathogens and microbes, applying tissue culture or micropropagation with an effective protocol can help increase ginger yield. The first and vital step for micropropagation is to select disease-free explants, sterilize and establish the culture and then induction of shoot and root multiplication, followed by acclimatization. *In vitro* propagation's most crucial function is to preserve genetic diversity and the evolutionary process in populations of ecologically and economically viable varieties and genotypes to prevent them from extinction [17].

Source and Type of Explants

According to the stages of a plant's development and environmental changes, the plant's physiological state changes spontaneously. Axillary buds and active shoot

CHAPTER 5

Micropropagation Protocol in *Atropa acuminata* Royle ex Lindl. and *Atropa belladonna* L.

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Abstract: *Atropa*, a Solanaceae member, contains many active chemical compounds such as atropine, saponins, polyphenols, scopolamine and hyoscyamine. Because of the presence of these active principles, endangered species *Atropa acuminata* and *Atropa belladonna* have been indiscriminately exploited in traditional medicine for treating various disorders and thus *Atropa acuminata* has become an endangered species in some regions. Due to the threat of extinction, low seed germination and seedling survival rate, there is a need for conservation through efficient micropropagation protocols. In this regard, the current chapter is focused on micropropagation methods/protocols developed by various researchers using various explants of *Atropa acuminata* and *Atropa belladonna* and their responses to different media compositions with respect to direct and indirect organogenesis *in vitro*, as the technique of *in vitro* regeneration has played a pivotal role in the mass multiplication of many plant species.

Keywords: *Atropa acuminata*, *Atropa belladonna*, Callus, Explants, Micropropagation.

INTRODUCTION

Atropa, a genus belonging to Solanaceae, is a toxic perennial herbaceous plant that prefers temperate climates and alkaline soils, often growing in light shade in woodland environments associated with limestone hills and mountains. It contains tropane alkaloids such as atropine, hyoscyamine, scopolamine, and others as medicinal compounds [1]. *A. acuminata* and *A. belladonna* are the most well known members of the genus *Atropa*. *A.acuminata* is a subalpine tall perennial plant native to Asia and found throughout the north western Himalayas [2]. *A. bel-*

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ladonna, also known as belladonna or deadly nightshade or poisonous plant [3], is native to Central and Southern Europe, North Africa, and Western Asia.

Tropane alkaloids and highly oxygenated triterpenes are active chemical compounds found in *A. acuminata*. Likewise, saponins, polyphenols, anthraquinones, phytosterols, tetrahydroxyoleane, oleanolic acid, and β -sitosterol are some of the other compounds found in *A. acuminata* [4]. Rhizomes of *A. acuminata* were used to treat arthritis, muscle and joint pain, and also muscle spasms [5, 6]. *A. belladonna*, on the other hand, contains belladonna alkaloids such as atropine, its isomer hyoscyamine and scopolamine, which are most important and used in medicine. Apoatropine, norhyoscyamine, belladonine, tropacocaine, noratropine, and meteloidine are some of the other alkaloids found in *A. belladonna* that are not widely used in medicine. *Atropa belladonna* has been used in folk medicine to improve wound healing, particularly in septic post traumatic wounds [7].

Atropine and hyoscyamine alkaloids from *Atropa* are used as an antidote to opium and as sympathetic nervous system stimulants. These are frequently used to control excessive salivation, sweating, and nasal secretions. Atropine has an effect on the respiratory and also on circulatory systems. Belladonna is used externally to treat neurologic pain and internally to treat asthma and whooping cough. Atropine is frequently used to treat muscle spasms (excessive muscular contractions) and in ophthalmologic examinations to dilate the eye pupil. Scopolamine, which depresses the parasympathetic nervous system, is used as a sedative or as an anti-insomnia medication. It is used in conjunction with morphine to induce twilight sleep. Tropane alkaloids have anticholinergic and spasmolytic properties, making them popular in eye surgery and as an anaesthetic and spasmolytic [8]. In traditional medicine, aerial parts of the *Atropa* plant are used to treat a variety of ailments including acute infections, anxiety, chicken pox, asthma [9, 10], acute inflammation, muscle and joint pain, peritonitis, pancreatitis, scarlet fever, neuroinflammatory disorders, Parkinson's disease [11, 12], conjunctivitis, and encephalitis fever [13, 14]. The root extracts were also used to treat sore throat, ulcerative colitis [15], as a sedative [16], and whooping cough [17].

Toporcer *et al.* [18] used *Atropa* for aseptic surgical skin wounds to differentiate between immunogenic and antibiotic effects, and they also demonstrated that *A. belladonna* has a positive effect on aseptic surgical wound healing. *A. acuminata* is a valuable medicinal plant due to the presence of tropane alkaloids such as atropine, hyoscyamine, and scopolamine, all of which have sedative, analgesic, mydriatic, antiasthmatic, anodyne, and antispasmodic properties [5, 19, 20]. The

main tropane alkaloids found in this plant are atropine and scopolamine, both of which have anticholinergic properties and are used in pharmaceuticals [21].

A. belladonna also contains tropane alkaloids and other active agents, such as atropine and hyoscyamine, throughout its plant parts. Because of this reason, it has been used for centuries in traditional treatments for a variety of conditions such as headache, menstrual symptoms, peptic ulcer, histamine reactions, and motion sickness, among others. Hyoscyamine, is also found in anti-vertigo medications and other medications that help to prevent motion sickness [22]. Atropine can also be used to treat hypertension and lower blood pressure [23].

IMPORTANCE OF MICROPROPAGATION IN *ATROPA*

A. acuminata is considered as an endangered species because wild plants are being indiscriminately harvested for their medicinal value from natural resources with no regard for cultivation practices [24 - 27]. Climate change, unplanned development, habitat destruction, excessive tourist traffic, and legal or illegal harvesting of this plant for local use and pharmaceutical industry needs are significant threats to its extinction. As a result of this, the IUCN has designated *A. acuminata* as an endangered species in the Kashmir Himalayas [28]. Due to the extinction threat, it is critical to develop necessary conservation strategies and efficient propagation protocols [29]. The low seed germination and seedling survival rate is a major constraint in traditional *A. acuminata* propagation methods [30]. As a result, developing an appropriate micropropagation protocol is critical for the survival of this critically endangered medicinal herb. Tissue culture has emerged as a broad field of study with tremendous potential for the conservation of endangered and rare plant species. Furthermore, tissue culture methods significantly increase the *in vitro* production of several bioactive compounds (non-enzymatic antioxidants) such as tannins, flavonoids, phenols, and so on, which significantly contribute to plant bioenrichment. Hence, *in vitro* regeneration techniques are used for many species of the genus *Atropa* [1, 31].

MICROPROPAGATION IN *ATROPA*

Explant Sterilization

Ahuja and co-workers used shoot tips and nodal segments of *A. acuminata* as explants, treating them with Tween-20 (Sigma, St Louis, Missouri, USA) for 10 minutes before thoroughly washing them with tap water for 30 minutes. The explants were then surface sterilized with 0.1% (w/v) mercuric chloride for 4 minutes after being soaked in 0.1% Bavistin for 10 minutes. To remove chemical residues, the explants were thoroughly washed 5 times with sterile distilled water [24]. Al-Ashaal *et al.* used leaves of *A. belladonna* as explants, washed them with

CHAPTER 6

Advances in Micropropagation Techniques of *Aegle marmelos* (L.) Corr.: A Review

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Abstract: *Aegle marmelos* (L.) Corr. is a plant of religious and medicinal importance in India. All of its plant parts have been reported to possess medicinal uses due to the presence of various phytoconstituents. Looking at its perspectives, *Aegle* is successfully propagated *in vitro*, primarily through organogenesis, using numerous explants. Efficient micropropagation is ensured by proper sterilization, preparation of explants, and use of antioxidants to avoid media browning. Various factors that affect the regeneration rate include season of explant collection, explant origin, phenological growth stage, concentration and combination of Plant Growth Regulators (PGR), culture media composition, and addition of additives to the media to enhance the micropropagation rate. The present review chapter compiles numerous reports of the effective micropropagation of *A. marmelos* and factors that affect the rate of micropropagation.

Keywords: *Aegle marmelos*, Bael, Micropropagation, Organogenesis, PGR.

INTRODUCTION

Aegle marmelos (L.) Corr, commonly called wood apple or stone apple, is indigenous to India. It is associated with spiritual and religious values. It belongs to the family Rutaceae. It is a slow-growing, spinous, tough, subtropical medium-sized tree. The fruits are used for both dietary and medicinal purposes.

A. marmelos contain several phytoconstituents namely marmenol, marmin, marmelosin, marmelide, psoralen, alloimperatorin, rutaretin, scopoletin, aegelin, marmelin, fagarine, anhydromarmelin, limonene, α-phellandrene, betulinic acid, marmesin, imperatorin, marmelosin, luvangentin and auroptene [1]. The Ayurvedic system considers it to be a healing tree that gives strength to the body, and this accreditation is due to its diverse medicinal properties considering all parts of this tree, namely, roots, leaves, trunk, fruit, and seeds, are used to cure va-

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rious human ailments and diseases [2 - 4]. Although all parts are useful, root and root bark are mainly used in medicinal preparations, which is why Bael suffers from destructive harvesting, as the tree has to be uprooted wholly to procure roots. Indiscriminate harvesting of the tree has posed so much threat that 'Red Data List of Indian Plants' has placed it in vulnerable threat status

MICROPROPAGATION

Aegle marmelos, a tree with immense medicinal importance, is traditionally cultivated through seed germination. Genetic variability, short viability, and vulnerability to insect and termite attacks make them unsuitable for propagation. Bael is also propagated vegetatively by root suckers, root cuttings, and layers, but the process is tedious and time-consuming [5]. The conventional plant propagation methods for woody trees are often difficult and slow because of high levels of heterozygosity and the long generation time between successive crosses.

Micropropagation is the only remedy that can help in overcoming these problems. In view of this scenario, a plethora of research work has been conducted on the morphogenic capacity of *Aegle*, and still, much more effort is required in this line [6, 7]. Trees have phenolic compounds; their life cycles are complex and long, are large sized, and therefore difficult to propagate. The intervention of modern propagation and improvement techniques for trees has become a necessity. Micropropagation using explants like shoot tip, root tip, shoot segments, nodal explants, cotyledonary nodes, zygotic embryos, *etc*., has been a boon in this direction. Plant tissue culture not only helps in the mass multiplication of superior genotypes but also is a basic requirement of transgenesis.

A large number of factors are responsible for the success of a plant tissue culture protocol like explants- type, age, physiological status of donor plant, type and concentration of growth regulators, carbon and nitrogen source, gelling agents and their concentration, and even method of explant sterilization. Suitable explant choice at a particular receptive stage, alteration in nutrient media composition, and growth additives could help lessen recalcitrance and give satisfactory micropropagation results. Different types of basal media like MS (Murashige and Skoog) [8], B5 medium (Gamborg Medium), WPM medium (Woody Plant Medium) were tested by Arumugam *et al.* [9]. Pati and Muthukumar [10] attained somatic embryogenesis and genetic transformation in *Aegle marmelos* using a half-strength (½) MS medium. Above all, regulated physical conditions like light, temperature, magnetic or electromagnetic fields, photoperiod [11], humidity, and other factors also play an important role in Bael tissue culture.

Proper sterilization and preparation of explants is a prerequisite step for the establishment of a successful *in vitro* plant regeneration protocol. The problem of

contamination is prominent in field-grown explants as compared to *in vitro* grown. There are several recommendations for decontaminating the explants before culture. For example, the use of teepol, a liquid detergent [12], NaOCl (Sodium Hypochlorite) [13], Clorox, Savlon [14, 15], or 5–10% Labolene [7, 16]. Many scientists have preferred using fungicides for disinfecting explants like Bavistin [12], Thiram, or 0.1% mercuric chloride ($HgCl_2$) [9, 15 - 22]. Princy *et al.* [11] were of the opinion that not only the concentration of $HgCl_2$ but the time duration of the treatment is also crucial for disinfection. Explant browning, necrosis, and decline in viability were noticed on increasing the time of sterilization from 5 minutes. A long period of surface disinfection is not suitable for juvenile explants such as shoot tips. Yadav and Singh [20] observed necrosis of shoot tips on being disinfected with 0.1% $HgCl_2$ for 8 minutes. They reported an optimal sterilization protocol for Bael tissue culture through experiments with elite varieties of Bael. According to them, soaking explants in 2.5% fungicide solution for two hours is the best for sterilization. Sometimes, a combination of antibiotics like rifampicin in combination with ethyl alcohol was also recommended [22].

Browning of cultured tissues due to the exudation of phenolic compounds from cut ends is another problem of concern in Bael micropropagation. To deal with this, antioxidants like citric acid, ascorbic acid, activated charcoal, and polyvinyl pyrrolidine (PVP) were added to the medium to avoid the browning of *A. marmelos* cultures [23]. Raj *et al.* [24] stated that the addition of activated charcoal in the medium for controlling browning intensity in cultures of Bael is unsurpassed.

Micropropagation in *Aegle* mainly relies on organogenesis using various explants, but somatic embryogenesis, androgenesis, and protoplast culture have also been explored, although not as successful as organogenesis [25].

ORGANOGENESIS

Correct choice of explants, explant origin, physiology of donor plant growth hormones, seasonal variations, *etc.,* affect organogenesis.

Explants

Different explants reported to demonstrate morphogenic potential in *Aegle* species are twigs [12], nodal segments [26], immature and mature leaf segments, tendrils, cotyledonary nodes [27 - 29], hypocotyls [22, 30], epicotyls [31], shoot tips [14], leaves from seedlings [9], root segments [9, 22], stem segments, *i.e.,* nodes and internodes [18, 32], nucellar tissue [33, 34], zygotic embryos [35], nodes from field-grown trees [7, 11, 12, 16, 21], nodes from root suckers [16], internodes or

CHAPTER 7

Biotechnological Aspects for Micropropagation of *Artemisia absinthium* L.

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Abstract: *Artemisia absinthium* L. or 'wormwood', commonly known as 'Dawna', is a small perennial herb with a dark fragrance due to glandular trichomes present all over the plant. Medicinal properties of *A. absinthium* are known in most of Asia, South America, and Europe. Essential oil, along with other phytoconstituents, like flavonoids, phenolic acids, tannins, and lignans, imparts medicinal potential to this species. It revealed antibacterial, antitumor, antimalarial, antioxidant, anthelmintic, antipyretic, antidepressant, antiulcer, antiprotozoal, hepatoprotective, neurotoxic and neuroprotective action. Due to its wide range of disease curing potential, *A. absinthium* germplasm is always under the pressure of overexploitation and loss of habitat. To cope with the higher industrial demand of this plant, the use of biotechnological techniques related to micropropagation can provide the best alternative. *In vitro* propagation using any explants has been extensively studied for the conservation of its plant genetic resources. Other micropropagation methods, such as callus culture, cell suspension, and organogenesis, have been adapted with the aim of secondary metabolite extraction and artemisinin enhancement. Modern biotechnological tools such as *Agrobacterium*-mediated gene transformation are mainly applied to hairy root and shoot cultures to optimize the biosynthesis of artemisinin. The present review throws light on various biotechnological studies carried out on *A. absinthium,* presenting the respective outcomes.

Keywords: Artemisinin, Biotechnology, *In vitro* propagation, Medicinal and Aromatic Plants, Micropropagation.

INTRODUCTION

Artemisia is an important genus belonging to the family Asteraceae (Compositae), having reputation for complex taxonomic problems. The generic name of this

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genus evolved from 'Artemis', a Greek Goddess, Diana [1]. Worldwide, the genus *Artemisia* is represented by more than 500 species, of which a maximum of 200 species were reported from China and 45 species documented in India [2]. In India, this genus was introduced around 800 A.D. by Arabian traders, amongst them *Artemisia absinthium* L. is used extensively in Materia medica [3]. *A. absinthium* is a perennial shrub measuring up to 80 cm in height, however at some locations, 1.5 m tall plants were reported [4]. The plant body is densely covered with hairs/glandular trichomes rich in essential oil, imparting a dense smell to the whole plant [5]. There are reports of 0.25-1.32% essential oils from stem and dry leaves of *A. absinthium* along with artemisinin, anabsin, artabsin, absinthin, matricin and anabsinthin. Compounds like flavonoids, phenolics, terpenes, and many other biologically active ingredients were profoundly found in *A. absinthium* [6, 7]. *A. absinthium* (Wormwood) is globally distributed in almost all continents. It was reported dominantly from Europe to North Asia, the Middle East, North and South America, and rare reports from Africa and Australia. In India, it is mostly confined to Kashmir Valley, Himachal Pradesh, and some hilly parts of South India above 1500 m [8]. In India, *A. absinthium* is known by many vernacular names like 'Afsanteen' in Urdu, 'Daman vishesh' 'Pranthaparna', 'Suparna' in Sanskrit, 'Saparna' 'Supreema', in Marathi, Majri, Karmala, Majtari, Mastiyarah in Hindi and as a 'Tethwen' in Kashmiri [1, 8]. As per medicinal importance, *A. absinthium* have antiulcer, neuroprotective, neurotoxic, anthelmintic, antiprotozoal, anti-inflammatory and anti-feedant properties [9 - 15]. Multiplication of *A. absinthium* is achieved by seed or by cutting and division, however, heterogeneous plantlets were seen in the seed germinated populations, and vegetative propagation is a quite slow process of multiplication [16]. Roots of *A. absinthium* are used for vegetative propagation. However, excessive irrigation damages the roots and leads to rotting [5]. In *A. absinthium*, true plant production is very critical as in field grown plants, biotic and abiotic stresses make plants more vulnerable [17]. Furthermore, ever-increasing demand for *A. absinthium* leads to overexploitation of its genetic resources leading to pressure on plant propagation [18]. To overcome this situation, *in vitro* propagation techniques provide an important alternative way. Micropropagation helps to produce infection-free genetically identical clones in very large numbers [19]. In recent years, *in vitro* propagation methodology has been extensively used for mass production and conservation of rare and endangered plant genetic resources [20]. The present review provides in-depth information on various biotechnological aspects successively used in micropropagation of *A. absinthium*.

BIOTECHNOLOGICAL ASPECTS OF *A. ABSINTHIUM*

Explants Sources

Leaves dissected from *A. absinthium* aseptic plantlets were successfully used for callus induction [21 - 25]. Foliage, stem, and nodal segment explants of *A. absinthium* were found effective in callogenesis and organogenesis [26 - 28]. Shoot induction through the callus of leaves was also reported by Koul and Lone [29] and Nin *et al*. [30]. Genetic transformation was quite successful in the callus cells grown from *A. absinthium* leaves and flowering tips [31], however, hairy root formation was achieved through aseptic shoots [32]. Soft tissues like shoot tips can be proliferated further in shoots and roots [33].

Culture Conditions

Light is an important physical factor that has the potential to modify plant architectural development [24]. The light intensity of 50 μmol m^{-2} s^{-1} photon flux density (PFD) for 16 h was applied by Koul and Lone [29]. In another study, Mannan *et al*. [34] used cool white fluorescent light of intensity 2000 lux for 16 h. While performing *Agrobacterium* mediated gene transfer, with cool white fluorescent light, 35 μmol m^{-2} s^{-2} was used [32]. A report of 40-50 μmol m^{-2} s^{-1} PFD light intensity for 12 h by cool white fluorescent was provided by Shekhawat and Manokari [27]. Cell suspension cultures placed at 40 μmol m^{-2} sec^{-1} revealed fluctuation in biomass [35]. The application of various spectral lights revealed morphogenic and biochemical variations in *A. absinthium* callus [24]. The cultures of *A. absinthium* were kept at 25 ± 1^0C [21]. Cell suspension cultures were also kept at 25 ± 1^0C [23]. Relative humidity was maintained at 60% [29]. pH of the culture medium was adjusted to 5.8 before heating using HCl or NaOH solutions, and the culture medium was subjected to autoclave for 20 min at 121°C and 108 kPa [33].

Tissue Surface Sterilization

In surface sterilization, initially *A. absinthium* explants were washed under running tap water to remove any surface attached material [21]. Mild detergent [26, 36] and Tween-20 (5% for 2 min) [33] were used to remove surface impurities from explants. Some researchers opt for antifungal agents like Benlate© solution. Lê *et al*. [37] washed leaf shoots with 0.1% Benlate© solution for 15 min. followed by soaking in 0.8% sodium hypochlorite for 15 min. In another experiment, Shekhawat and Manokari [27] used 0.1% aqueous solution of Bavistin for 5 min as a systemic disinfectant, followed by 0.1% mercuric chloride treatment. Explants were exposed to 70% ethanol for 30 minutes, followed by 0.01% mercuric chloride solution, later rinsed with sterile distilled water [19, 29,

CHAPTER 8

Conservation of Medicinal Plant Bramhi- *Bacopa monnieri* (L.) Wettstein Through *in vitro* Cultures

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Abstract: *Bacopa monnieri* (L.) Wettstein is a medicinal herb from the family Plantaginaceae widely known as 'water hyssop' or 'brahmi'. The therapeutic potential of plants is due to the presence of many bioactive secondary metabolites, majorly brahmine, herpestine, alkaloids, and saponins (bacosides), which are responsible for pharmacological effects including neuroprotective, hepatoprotective, gastroprotective, antioxidant, anti-inflammatory, and antimicrobial properties. Vegetative cultivation of *Bacopa* on a large scale has its limitations due to the lack of viability of seeds during propagation and the unpredictable nature of the production of phytochemicals for commercial purposes, which can be overcome by tissue culture mechanism. Over the past few decades, many studies on the tissue culture of *Bacopa* in establishing a standardized protocol were reported. This chapter deals with *de novo* organogenesis of the root and shoot along with the callus induction and somatic embryogenesis from different explants of *B. monnieri* on MS basal nutrient medium supplemented with Plant Growth Regulators.

Keywords: *Bacopa monnieri*, Micropropagation, Plant growth regulator.

INTRODUCTION

Bacopa monnieri, a renowned medicinal herb, belongs to the family Plantaginaceae. The plant is a short-lived annual herb that is common in wet habitats and along waterways, and originates from tropical and subtropical Asia. It thrives on plains and slopes near flowing water and wetlands and is especially prolific during monsoon [1]. As an aquatic herb, it has been utilised both for therapeutic use and as a decorative plant in aquariums and ponds. It thrives in ma-

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rshy environments, lakeshores, shorelines, and seaside locations along canals and channels, where the formation of dense mats is frequently seen [2].

It can be found all over the world, including in Asia, Africa, the Americas, Australia, the Eastern Mediterranean, the Arabian Peninsula, and the Caribbean [2 - 4]. It is reported as having been introduced into Japan, Singapore, Spain, Portugal, Andaman Islands, California, Marquesas, and Cayman Islands, among other countries [2, 5 - 9]. *Bacopa monnieri* is most popularly known as Brahmi, water hyssop but has many international and local common names because it is widely distributed all over the world, such as coastal water-hyssop and herb-of-grace [2]. According to the Indian Medicinal Plants Database, there are 183 vernacular names for *B. monnieri* in 9 different languages in India.

Many Ayurvedic remedies, including Brahmi rasayana, Brahmivati, Brahmighrit, and Sarasvatarisht, use *B. monnieri* as a useful ingredient [10]. Ayurveda considers *Bacopa* as a booster for the nervous system, which aids in enhancing memory, focusing and treating mental disorders [11, 12]. An important traditional Medhya Rasayana medicine in Ayurveda is made from *B. monnieri* [13]. Both children's and teenager's cognitive and behavioral traits were enhanced by the herb [14]. Besides, plant exhibits anti-cancer, analgesic, antioxidant, antipyretic, pro-cognitive, neuropsychiatric, neuroprotective, anti-neuroinflammatory, anti-inflammatory, anti-bacterial, anti-fungal and anticonvulsant properties [13, 15 - 27].

The plant is used in India and Pakistan for gastrointestinal stimulation, a heart restorative, and to assist respiratory function during bronchoconstriction [28]. In the Indian Materia Medica and Traditional Chinese Medicine, *B. monnieri* has been recommended as a treatment for several mental health issues, including insomnia, psychosis, anxiety, impaired cognition, and depression [29 - 31]. Additionally, the plant extract provided defense against opioid and tacrolimus-mediated renal damage [32, 33]. Furthermore, the plant has been shown to have anti-anhedonian, vasodilator, hippocampus-strengthening, anti-cytotoxic, anti-genotoxic and hormetic properties [21, 34 - 36].

In *B. monnieri,* important bioactive compounds categorized under tannin, phlobetannin, saponin (bacosides A and B), steroid, flavonoid, cardiac glycoside, phenol, and alkaloid (nicotine and herpestine) were reported under phytochemical screening investigations [27]. The presence of numerous triterpenoid saponins, including bacosides A, B, C, and D, often known as "memory chemicals," has been linked with the pharmacological properties of *Bacopa* in improving comprehension and remembrance [37 - 39].

Due to the high therapeutic potential and wide usage of *B. monnieri,* it should be cultivated separately as the plant propagates vegetatively and reproduces asexually, which makes it difficult to recognise or detect in the field. Despite its rapid growth, invasiveness into the native environment, adaptability, and toleration under abiotic stress, it negatively impacts aquatic habitats by causing damage to the flora and fauna along with the water. In terrestrial environments, it destroys stream banks, wetlands and lakeside edges, and shorelines [2].

It can grow as a weed in rice fields and beneath date palms for small-scale irrigated fields [3, 8, 40], which does not compensate for structured mass cultivation. Apart from these, biotic or natural enemies for plant include *Anartia jatrophae*, the white peacock butterfly, that feeds its caterpillars on *B. monnieri*. Damage from the tobacco cutworm *Spodoptera litura* has been noted in greenhouse setups [41]. Several nematodes belonging to the genus *Meloidogyne* also live on *B. monnieri* as a host [40], which are major threats for the plant under its natural habitat. Because of their limited vitality (two months), *B. monnieri* seeds are regarded as poor propagules, and the seedlings frequently wither during the development of the secondary node which makes it challenging to grow from seeds. The whole plant is utilised for a variety of medical applications, creating a commercial demand for it that leads to the exploitation of plant species by various enterprises, including the adulteration of plant products [42].

Plant tissue culture offers an alternative approach for cultivation and conservation through *in vitro* propagation, which is much appropriate and sustainable compared to other methods. Additionally, it provides adequate samples for further research and development to explore a variety of additional advantages of *B. monnieri*, as well as contamination-free, carefully chosen plants of true type with quantification of desired phytochemicals that can meet industrial demand in the preparation of drugs.

MICROPROPAGATION OF *BACOPA MONNIERI*

Micropropagation of *B. monnieri* requires a stepwise protocol which includes,

Source and Selection of Explants

For direct organogenesis of shoots, the use of microshoots, leaves, or internodal explants removed from the base of the plant *B. monnieri* has been proven to be particularly efficient [43]. Additionally, direct somatic embryos were discovered utilizing leaf explants made from microshoots [44]. For the establishment of callus cultures, axillary buds, younger nodes, shoot tips, and young leaves cut from the young shoots have been employed [45, 46]. It was also reported that for the generation of bioactive compounds like bacosides, nodal parts and leaves were

CHAPTER 9

A Review of *Momordica charantia* L.: Regeneration *via* Organogenesis *versus* Embryogenesis

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Abstract: *Momordica charantia* L., commonly known as bitter melon/gourd, is a slender tendril-climbing annual vine of the family Cucurbitaceae. Bitter melon grows in tropical areas, including parts of the Amazon, Asia, and the Caribbean, and is cultivated throughout South America. It is a common food of the tropics used in the treatment of many diseases and is also known for its potent hypoglycemic actions. A steroidal sapogenins known as charantin, insulin-like peptides, and alkaloids have been reported to have hypoglycemic or other actions of potential benefit in diabetes mellitus. The present chapter gives a comprehensive review of the tissue culture of *Momordica charantia*. There are two ways of regeneration, direct organogenesis and indirect organogenesis; both take place through the production of adventitious buds and somatic embryogenesis. The present review gives a complete *in vitro* regeneration protocol of *M. charantia*.

Keywords: Clonal propagation, Differentiation, Explants, *In vitro* regeneration, Organogenesis, Regeneration, Somatic embryogenesis.

INTRODUCTION

Momordica charantia L., commonly known as bitter melon/bitter gourd, is a tendril climbing plant belonging to the family Cucurbitaceae. It is distributed throughout the globe with predominance in tropical and subtropical areas [1, 2]. It is widely grown in India and other parts of the Indian subcontinent, Southeast Asia, China, Africa, the Caribbean, and South America for food and medicine [3]. Fruits of *M. charantia* are used as daily food, whereas the fruits, leaves, roots, and seeds of bitter melon are used as traditional medicine in Southeast Asia and Indo-China [4]. *M. charantia* has culinary use and is also a vegetable [5, 6]. *M. charantia* possesses many biological activities. Roots are acrid, astringent, and bitter, and leaves are antipyretic, emetic as well as purgative and bitter. Fruits are

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acrid, anthelmintic, anti-diabetic, anti-inflammatory, appetizer, bitter, depurative, digestive, purgative, stimulant, stomachic, and thermogenic [7]. Momordicin, a compound of bitter melon, gives the plant a characteristic of bitter taste and also has stomachic properties [8].

Momordica charantia has a very important place in Ayurveda and is recommended for treating many diseases like anemia, bronchitis, blood diseases, cholera, diarrhea, dysentery, fever, hepatitis, itch, ulcer, measles, and sexual tonic and as a cure for gonorrhea [9]. Fruits are used as a traditional medication to cure various diseases like rheumatism, gout, worms, colic, diseases of the liver, and spleen [10]. It is also found useful in the treatment of cancer and diabetes mellitus [11].

It contains an array of biologically active chemicals, including triterpenes, proteins, steroids, alkaloids, saponins, flavonoids, and acids due to which plant possesses anti-fungal, anti-bacterial, anti-parasitic, anti-viral, anti-fertility, anti-tumorous, hypoglycemic, and anti-carcinogenic properties [12 - 16]. This plant is blessed with therapeutic potential [2, 17 - 19], such as antioxidant [20 - 23], antimicrobial [2, 17, 21, 24 - 26], anthelmintic [27, 28], anti-diabetic [2, 20, 29, 30], anti-inflammatory [30, 31], antihyperglycemic [2, 30, 32], anticancer [4, 28, 30, 33], antimicrobial [43] and nutritional as antilipolytic [20, 34] due to the presence of bioactive compounds. It is a potent hypoglycemic agent due to alkaloids and insulin-like peptides and a mixture of steroidal sapogenins known as charantin [32].

Chemical Composition

Chemically, the plant is enriched with secondary metabolites like triterpenoids [20, 35-37], saponins [38], polypeptides [39], flavonoids [23, 40, 41], alkaloids [42, 44] and sterols [22, 45 - 47] which are distributed throughout the entire plant. The seed is not edible; it contains extractable oils, mostly a conjugated triene *cis*-9, trans-, trans-ctt [9, 11, 13], and conjugated omeroflinolenic acid, known as α-elestearic acid (α-ESA). The anti-cancer and anti-obesity properties are due to the presence of ESA [48], while phenolic compounds phenylpropanoids, flavonoids, triterpenes, and carotenoids [40, 49] are responsible for pharmaceutical properties. The main bioactive compounds of the fruit of *M. charantia* are carbohydrates, proteins, lipids, and more [35, 47, 50].

Antimicrobial properties of the plant are due to the presence of charantin which is a Cucurbitane-typetriterpenoid [51]. Charente is a mixture of two steroidal saponins, β-sitosterol glycoside and stigma sterol glycoside [52]. Charantin is present mainly in fruits, leaves, and roots [27, 35, 36, 44]. However, cucurbitane-type triterpenoids are found in the entire plant. Antimicrobial activity is also

linked to [53, 54] α-momorcharin (leaf and seed) and MAP30 (fruit and seed). MAP30 and α-momorcharin show antimicrobial properties as they are ribosome-inactivating proteins [35, 54].

PLANT TISSUE CULTURE OF *MOMORDICA CHARANTIA* L.

Initiation of adventitious buds and somatic embryo formation are two ways of regeneration [55]. Callus formation is an important stage in the plant regeneration process as the callus can give rise to shoots, roots, and both roots as well as multiple shoots. The process of regeneration mediated by callus is also known as indirect regeneration.

Callus Formation in *Momordica charantia* L.

Thiruvengadam *et al*. [55] reported about the callus formation in *M. charantia*. They stated that in Murashige and Skoog (MS) medium supplemented with 1.0 mg/l 2,4-Dichlorophenoxy acetic acid (2,4-D) well-organized friable calluses were formed by approximately 90% of leaf explants. Compact and hard green calluses were produced at various concentrations of hormones like 6-Benzy--aminopurine (BAP) and Kn, which turned embryogenic due to plant growth regulator stress. BAP is useful for the development of good texture callus [56]. In *Momordica dioica,* 1.0 mg/l BAP + 0.1 mg/naphthalene acetic acid (NAA) combination produced soft, green, light, and friable calli [57]. The greenish compact callus was produced on the combination of 7.7 μM NAA + 2.2 μM Thiadiazuron (TDZ) from leaf explants of *M. charantia*.

Indirect Organogenesis

Indirect Organogenesis through Somatic Embryos

Thiruvengadam *et al.* [58] developed a system for the somatic embryogenic suspension culture of *M. charantia*. They reported the formation of friable calluses in 30-day-old leaves on semi-solid MS medium [59] supplemented with 1 mg/l 2,4-D. On sub-culturing the callus in liquid medium with 1.5 mg/l 2,4-D, a large number of globular embryos (about 24.6%) was noticed and by removing 2,4-D during later stages, they turned to heart/torpedo stages. The germination of these embryos was on basal MS media. They studied the effect of media, carbohydrates, and amino acids on somatic embryogenesis *via* the formation of cell clusters, which then enlarged to pro-embryos, and gave rise to mature embryos within a period of 2 weeks. A high-frequency induction, maturation, and development of somatic embryos were on MS medium with 50 mg/l polyvinyl pyrrolidone (PVP) and 40 mg/l glutamine. About 6.2% phenotypically normal young plantlets were developed from friable callus.

CHAPTER 10

In Vitro Protocols for Micropropagation of *Catharanthus roseus* (L.) G. Don

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Abstract: *Catharanthus roseus* (*C. roseus*) is an important alkaloid-yielding medicinal and ornamental plant belonging to the family Apocynaceae. The genus *Catharanthus* is well studied and reported to contain biologically active terpenoid indole alkaloids (TIAs) with over 130 compounds isolated and identified. It has great medicinal importance in treating various ailments to treat diseases as diabetes, malaria, menorrhagia, Hodgkin's disease, *etc*. In view of the immense importance in the pharmaceutical industry, micropropagation of *C. roseus* has been the best alternative for continuous source of plants and also for *in vitro* production of secondary metabolites. Various explants have been studied for micropropagation; however, nodal explants were the most suitable. For surface sterilization, 0.1% $HgCl_2$ or 70% ethanol, followed by sodium hypochlorite and Bavistin (carbendazim), was optimum to control the microbial contamination. Murashige and Skoog (MS) medium was the most widely used for its success rate. 2,4-D for callus initiation and BAP, along with zeatin and activated charcoal, were reported to be promising for regeneration of plantlets. The 100% acclimatization of plantlets on transfer to field depends on the soil mixture and environmental conditions and humidity in the initial stages of transfer from *in vitro* cultures.

Keywords: Acclimatization, *Catharanthus roseus*, *In vitro* Studies, Micropropagation, Organogenesis, Plant Regeneration, Somatic Embryogenesis.

INTRODUCTION

Catharanthus roseus (L.) G. Don, commonly known as Madagascar periwinkle, is an important medicinal plant. It belongs to the family Apocynaceae. It contains several commercially valuable secondary metabolites, making it the most demanding medicinal plant. The secondary metabolites are used in the treatment of various ailments and disorders like Hodkin's disease, lymphoblastic leukaemia, breast and skin cancer and cancerous tumours [1]. The periwinkle has been repor-

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ted to contain a good source of commercial bioactive alkaloids, including vinblastine and vincristine, which have anti-cancer activities. *C. roseus* plant has been reported to contain several other important bioactive compounds, such as anthocyanins, flavanol glycosides, phenolic acids, saponins, steroids, and terpenoids, that exhibit antidiarrheal, antidiabetic, anti-hypoglycaemic, antimicrobial, wound healing, and antioxidant activities, respectively [1, 2].

The leaves of *C. roseus* contain secondary metabolites (vindoline, vinblastine, catharanthine, vincristine, *etc*.), whereas in stem and roots, ajmalicine, reserpine, serpentine, horhammericine, taborsonine, *etc*., are present. Two alkaloids vincristine and vinblastine possess anticancer properties. Hence, it is used immensely in the pharmaceutical industry. The alkaloids of *C. roseus* comprise a group of about 130 terpenoid indole alkaloids (TIA). Vinblastine has now marketed for more than 40 years as an anticancer drug and has become a true lead compound for drug development [2]. Due to its low volume with high value in pharmaceutically important drugs, *in vitro* studies are the best alternatives for the production of these alkaloids. The conventional methods of propagation through seed germination were not encouraged due to its low germination rate (30%) and low vigour [3]. Plant tissue culture technology is a promising method to produce true-to-type plants without destroying the plant. Many researchers tried and tested various plant tissue culture techniques for clonal propagation on a large scale and also to improve the alkaloid content of *C. roseus*.

IN VITRO STUDIES IN *CATHARANTHUS ROSEUS*

The Explants

A large number of plants can be developed in a short period in a small space under controlled and aseptic conditions *via* micropropagation. The selection of explant plays an important role in plant tissue culture. Various explants of *C. roseus* have been used, such as nodal segment, axillary bud, shoot tip or apical bud, leaf, stem, anther, petiole, root, *etc*. The choice of explant is based on various criteria such as availability of material, response and objective [4]. With respect to optimum production of shoots and roots *in vitro*, the nodal segment (node) was most responsive [5, 6]. Apical meristem or shoot tip consisting of apical or axillary buds also proved to be a quick responsive explant for direct organogenesis [7]. Verma and Mathur [8] produced adventitious shoot buds and roots using *in vitro* grown leaf explants. Studies reported the use of hypocotyl, anthers and zygotic embryos as explants for callus induction and regeneration produced embryogenic callus from anthers [9, 10], however, hypocotyl explant was reported to be promising for somatic embryogenesis [11]. Leaf petiole when used as an explant produced callus and roots [12]. Epicotyl with increased

vincristine with shooty teratoma was reported by Begam *et al.* [13]. Due to availability of lateral meristem, the nodal segments may be the best explants for mass multiplication through direct organogenesis [13, 14].

Surface Sterilization

The selection of surface sterilants is another crucial step for the establishment of aseptic cultures *in vitro*. It is a well-known fact that the concentration and the duration of treatment with sterilant vary for different species and explant to explant besides the load of contamination in the explant. The use of 70% (v/v) ethanol wash in *C. roseus* for 30 seconds to 1 minute, along with other sterilants, was found to be very common for surface sterilization of explants. However, some researchers reported to use higher concentration of ethanol (75-90%) and up to 2 minutes with 70% ethanol [15]. Ethanol wash is followed by treatment with commercial bleach or sodium hypochlorite (0.1–25%), and the duration of treatment was 5–45 min and sometimes along with a few drops of liquid detergent Tween-20 (polysorbate 20) or Tween-80 (polysorbate 80) or Triton-X was also reported.

The explants of *C. roseus* were also treated with mercuric chloride. Even though mercuric chloride is toxic to plants, it has been used by researchers in the range of 0.04–0.5% (w/v) for 2–5 min, maximum up to 15 min [16]. Besides, the explants were also treated with hydrogen peroxide, Labolene (Qualigen- Fisher, LR grade), Teepol (Reckitt Benckiser Pvt. Ltd., India), Dettol, *etc* [6]. In order to control contamination further with fungal species, researchers also treated the explants with fungicide Bavistin (Crystal crop protection Ltd., India) and/or antibiotic solution (Cefotaxime or Streptomycin). To summarize, the explants treated with multiple surface sterilants starting from 70% ethanol treatment followed by NaOCl along with detergent, Bavistin, mercuric chloride and sometimes 5% H_2O_2 were found to be more effective in preventing and controlling microbial contamination in *C. roseus* cultures [17].

Culture Medium

The selection of a suitable basal medium depends upon the objective and the type of plant species used in the experiment [18, 19]. Murashige and Skoog (MS) medium [20] is the most commonly used basal medium to this day and is reported to be also responsive to culturing of *C. roseus*. For organogenesis, a full-strength MS medium was utilized, reported to provide the required nutrients for shoot bud induction and shoot proliferation (Table **1**). Half strength MS medium was reported to be used for root induction in *C. roseus* [13, 14]. Other media such as woody plant medium (WPM) [19], Gamborg's B5 medium [21], and liquid Linsmaier and Skoog [22] medium were also tested.

CHAPTER 11

A Systematic Review on Micropropagation of Medicinal and Vulnerable Ashoka Tree [*Saraca asoca* (Roxb.) W.J.de Wilde]

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Abstract: *Saraca asoca* (Family - Fabaceae) is well-known medicinal tree species used in codified and non-codified systems of traditional medicine. Tree parts, *viz*., bark, flower, leaf, root and fruit, are used to treat various disorders. A huge number of pharmaceutical products were prepared using bark as one of the major ingredients. Ashoka tree is categorized as vulnerable by the International Union for Conservation of Nature (IUCN) and endangered by the Conservation Assessment and Management Plan (CAMP) due to its overexploitation and limited distribution. Unsustainable utilization, deforestation and climate changes are the major threats to the existence of the species. Aiming at the conservation of the Ashoka tree, ample research works were performed to standardize the micropropagation techniques. The present chapter discusses the efforts made towards conservation and micropropagation studies on the Ashoka tree.

Keywords: Ayurveda, Conservation, Medicinal Plant, *Saraca asoca*, Tissue Culture, Vulnerable.

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INTRODUCTION

Saraca asoca (Roxb.) W.J.de Wilde is an evergreen small-sized tree that belongs to the family Fabaceae [1]. It is popularly known as the Ashoka tree and other vernacular names of this tree in different languages of the Indian subcontinent are Sitaashoka (Sanskrit, Nepali, Hindi, and Gujarati), Aachange, Seeta ashokada mara, Eliyala, Kenkali mara, Kempuchinnada ele gida (Kannada), Asokamu (Telugu), Hemapushpam (Malayalam), Jasundi (Marathi), and Asogam (Tamil) [2]. It is native to the Indian subcontinent, and the scattered populations are found in the Indo-Malayan (from Pakistan to Malaysia) region. In India, it is mainly found in Eastern and Western Ghats, sub-Himalayan tracts from Uttar Pradesh to Eastern states of India [3 - 5]. Socio-culturally, it is a valued tree in India and elsewhere and occupies a privileged place in Hindu tradition. The term Ashoka means 'without sorrow' or 'the one which takes out the sorrow' [3]. The tree was mentioned in the ancient Indian treatise, the '*Ramayana*' [4].

Medicinal Uses

The tree is used to cure various disorders in codified (Ayurveda, Unani, Siddha) and non-codified folklore systems of traditional medicine. Bark and bark products are chiefly used to treat various gynecological disorders and other conditions (Table **1**) [4 - 9].

Table 1. Medicinal uses of *S. asoca* bark.

Gynecological Disorders	Other Medicinal Uses
Excessive bleeding, stress, gynecological disorders, irregular menses, premenstrual syndrome, ovarian cysts, fibroids, dysfunctional uterine bleeding, menstrual flow issues, uterine inflammation, menopause-related indications, menorrhagia, dysmenorrhea, leucorrhea, metrorrhagia, menopausal syndrome, postmenopausal syndrome, premenstrual tension, genitourinary diseases, pubertal and menopausal bleeding, spasmodic and lower back pain, stress and mood swings.	Fever, neurological disorders, snake bites, disease of the eye, wounds, skin diseases, including leprosy, anti-abortion agent, anemia, improved skin complexion, piles, burning sensation, tumors, dermatitis, cure indigestion, animal bite, hair tonic, biliousness, dyspepsia, dysentery, colic, piles, ulcers and pimples.

Folklore healers suggest bathing under the shade of the Ashoka tree for patients suffering from mental disorders. According to the local practitioners, mental peace or mental stability can be obtained by wearing lei (*Maala*) using root pieces of the tree. Further, the leaf, inflorescence, root, fruit, seed and whole plant parts were also reported to be used in the treatment of blood purification, dysentery, diabetes, menorrhagia, bleeding piles, dysentery, kidney stones, cough, and to prevent abortion, as cardiotonic and cooling agent [4, 6 - 8].

Phytoconstituents and Bioactivity

It is reported to contain a number of bioactive constituents in Ashoka tree such as catechin, leucocyanidin, epicatechin, procyanidine B-2, saracoside, β-sitosterol, lignin glycosides, procyanidin gallate, myoinositol, oleic, linoleic, palmitic and stearic acids, quercetin, kaempferol-3-O-P-D-glucoside, *etc*. These phytochemical constituents are responsible for antioxidant, antibacterial, antifungal, anticancer, antiulcer, analgesic, antiarthritic, anti-inflammatory, anti-nephrolithiatic, antide-pressant, antidiabetic, hypolipidemic, larvicidal, antimutagenic, antimennorhagic, oxytocic, genoprotective and uterine tonic properties. It also acts cardioprotective, dermatoprotective and brain tonic agent [3 - 6, 8, 9].

Conservation Status

The bark of the tree is the main part harvested from the wild populations, and the market demand for bark is about 15,000 metric tons during 2007–2011. It is increasing over the time by crude drug market and pharmaceutical industries. Flowers and leaves were also reported to be marketed locally in India at a smaller scale [4]. Unsustainable harvesting of bark in large volumes from wild populations is one of the main reasons for the rapid depletion of the plant along with its sensitive niche. Habitat destruction, forest fire, encroachment, domestic animal grazing, developmental activities and changing climatic conditions are the other reasons for the rapid decrease in its natural habitat. Hence, the tree species is listed in the Rare, Endangered and Threatened (RET) category of vulnerable status by the International Union for Conservation of Nature (IUCN, 2022) [10] and endangered by Conservation Assessment and Management Plan (CAMP, 2001) [5, 11]. Further, fair to poor natural regeneration status was also reported for *S. asoca* [9]. Therefore, several efforts were made to develop *in vitro* tissue culture techniques to enhance the regeneration of the plant and ultimately to achieve the conservation strategies and action plans. The present chapter emphasizes the ample research works carried out on micropropagation studies of the endangered medicinal plant *S. asoca*.

Reproductive Biology

Ashoka tree is a habitat specific evergreen perennial tree that produces bright colored fragrant flowers in paniculate corymbose inflorescence during the month of December to May. The flowers change their color from light orange/yellow to scarlet from their initiation to wilting. The pollination is entomophilous, and the successful anthesis for cross pollination and pollen germination was reported in the early morning periods. The pods mature from May to July, however, variations in phenology were observed in the plants grown in different locations [12]. Seeds are recalcitrant, and seed germination studies showed physiological

CHAPTER 12

In Vitro Propagation of Yam as a Medicinal Plant

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Abstract: Yam (*Dioscorea* spp.), a tropical monocot flowering, perennial multi-species crop, belongs to the family Dioscoreaceae. It is a valuable source of medicines and food security crops in yam-growing regions in Asia, Africa, and southern American countries. More than 600 yam species are widely cultivated in tropical and subtropical countries and used as food and medication for various human diseases. It provides big starchy tuberous roots as a source of carbohydrates, protein, antioxidants, minerals, and vitamins. It is also high in vitamin C, B6, manganese, potassium, and antioxidant compounds, which nourish and protect against oxidative cell damage in the human body. In addition, they are rich in potent plant compounds, including anthocyanins, a color-producing chemical that helps to reduce blood pressure and inflammation and protect against cancer and diabetes. Exceptionally, yam is an excellent crop for food security and human health. Micropropagation of medicinal yam is essential for the large-scale multiplication and conservation of endangered species. So far, in micropropagation of medicinal yam spp., very few studies have been conducted. These studies used axillary buds, nodal cuttings, mature, immature leaves, and shoot tips as explants for micropropagation. Several tissue culture techniques are available for micropropagation of yam, especially direct and indirect organogenesis for *in-vitro* propagation for large-scale generation of plantlets.

Keywords: *Dioscorea* spp., Medicinal yams, Micropropagation, Organogenesis, Regeneration, Tissue culture, Yam basal medium.

INTRODUCTION

Origin, Cultivation Classification, Economic Value, Production

Yam (*Dioscorea* spp.), a tropical monocot flowering, perennial, and multi-species crop, belongs to the family Dioscoreaceae. It produces edible large-sized tubers for food as well as medicine. It possesses creeper plant-like stems with heart-shaped green leaves and white or green flowers and berry-like fruits. Yam is the third most important root and tuber crop next to cassava and sweet potato [1]. It is

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a staple food as well as full of medicinal qualities, grown in several West African countries since 11,000 BC [2], mainly in Nigeria, Ghana, Côte d'Ivoire, Benin, and Togo, which are known as yam-belts in Africa that is responsible for more than 92% of the total yam production [3]. In the region, yam cultivation covers over 8.1 million hectares, with a total annual production of over 67 million tons [4]. Ghana and Nigeria alone account for 77% of the product of yam. The crop also contributes much more protein to the human diet than the more widely grown cassava and even more than meat protein [5].

Medicinal Importance of Yam

More than 600 yam varieties are available globally in the genus *Dioscorea,* and only twelve yam varieties are extensively disseminated in the growing region, especially in Africa, Asia, Oceania, and South America. Other yam varieties are grown as wild plants in nature. These wild plant varieties are bitter and full of medicinal compounds. Waris *et al.* reported that the tuber, leaves, and stem of *D. deltoidea* are used to treat jaundice [6]. Tubers of *D. dumetorum* are used as a birth control agent for controlling the human population [7]. In 2018, Mustafa *et al.* [8] reported that the tuber and leaves of *D. belophylla* are used to treat malaria, jaundice, and dysentery. It contains exceptional medicinal properties enriched with alkaloids and steroids [9, 10]. Plant-based medicine has been used for centuries as an alternative medicine for various human diseases, mainly menopausal symptoms, diabetes, rheumatoid arthritis, and muscular cramps [11]. The tubers of wild yams contain a chemical compound called diosgenin, which can produce various essential hormones in our body, especially estrogen [12]. Traditional healers in West African countries use wild yam tubers as an alternative to hormone replacement therapy during menopausal conditions of older women. Various formulations like tablets, capsules, powders, tinctures, and creams are commercially available. Yam tubers also contain a chemical known as dioscoretine, which regulates blood sugar levels to the optimal range in the animal model study [13]. It needs to confirm further research and validation on human research. It is also helpful in chronic joint pain, rheumatoid, and several muscular cramp-related disorders [14]. Some species contain vitamin C and B6, manganese, and potassium [15]. The African yam (*Dioscorea* spp.) contains thiocyanate, which can potentially protect against sickle cell anemia [16]. Tubers of certain wild species of *Dioscorea*, such as *D. nipponica*, were found to contain diosgenin, a steroid sapogenin extracted and used for the commercial synthesis of cortisone, pregnenolone, progesterone, and other steroid products [17]. Wild yam tubers have been reported to be a preventive or therapeutic medicine against several ailments, including arthritis, cancer, diabetes, gastrointestinal disorders, high cholesterol, and inflammation in Memorial Sloan-Kettering Cancer Center [18]. The Chinese medicinal yams are known for their high therapeutic value to human

health, which can be used to treat chronic diarrhea, chronic enteritis, spleen malfunctions, lung infections, gastric diseases, diabetes, nocturnal emission, enuresis, and underlying embolism [19, 20]. Yams have also been used as healthy food and herbal medicinal ingredients in traditional Chinese medicine [21]. In humans, Yam extracts showed significant antioxidant activity and modified serum lipid levels [22]. Yam flour was reported to protect rats from chemical-induced toxicity [23]. Several previous researchers report the medicinal properties and uses of different species of medicinal yams. Pillai *et al.* reported that the starch content of *D. esculenta* makes it viable for therapeutic purposes [24]. In 2021, Parida and Sarangi reported that tubers of *D. glabra*, *D. puber*, and *D. wallichii* have many medicinal uses. In traditional Chinese medicine, a decoction of pieces of yam is also a popular method of consumption [25].

D. alata, also known as purple yam, looks peculiar and contains high nutritional content. The flesh of this yam is purple and has a potato texture when cooked. Many people like its sweet and nutty flavor. It can be cooked in a variety of ways. Apart from their taste, purple yams are also a rich source of antioxidants, vitamins, and minerals. It is also high in vitamin C and can increase antioxidant levels by up to 35%, protecting against oxidative cell damage. In addition, they are rich in potent plant compounds and antioxidants, including anthocyanins, which give them their lively color. Studies have shown that anthocyanins may help reduce blood pressure and inflammation and protect against cancer and type 2 diabetes [26, 27].

Yam as a Staple Food

Yam is a tuber-producing crop that serves as a valuable food source in tropical and sub-tropical countries across Africa, Southeast Asia, South America, the Caribbean, and the Pacific islands [28]. Yams are used not only as fresh vegetables but also as processed foods like chips, dry roasted slices, flakes, flours, fried in oils, grilled, baked, pounded paste (fufu), and barbecued. It can be cooked with rice, plantain, beans, sweet potato, lamb, chicken, and butternut as squash soup [29]. The significant challenges in yam production can be categorized into several biotic and abiotic factors, including lack of clean, disease-free planting material, pests, diseases, decreasing soil fertility, and yield potential [30, 31]. Climate change has a significant impact on crop phenology, like the formation of authentic medicinal materials. Several studies reported [32 - 34] that authenticity is the core symbol of quality restorative materials, especially in Chinese medicinal yams. The specific locations of medicinal yams have been a manifestation of their prominent geographical characteristics, which are closely related to their demands for unique climate, soil, and other ecological conditions [35].

CHAPTER 13

Micropropagation of the Medicinal Plant 'Sarpagandha' [*Rauvolfia serpentina* (L.) Benth. *ex* Kurz] and its Applications in Human Welfare

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Abstract: *Rauvolfia serpentina* (L). Benth. ex Kurz., commonly known as Sarpagandha (Indian snakewood), of the family Apocynaceae, is a medicinally important woody shrub. Since ancient times, the root of this shrub has been used for treating numerous diseases, especially hypertension, mental agitation and cardiovascular diseases. In addition to eighty different alkaloids, all well-known for their pharmaceutical properties, the plant also contains reserpine, recognized as the world's first antihypertensive drug. Thus, the demand for this plant has only grown in the pharmaceutical industry. However, overexploitation and abysmal traditional propagation methods have endangered this valuable species' natural vegetation, creating an unpleasant gap between the demand and availability. In this scenario, the *in vitro* micropropagation technique comes as an alternative strategy to help replenish this threatened shrub's natural vegetation loss and commercial needs. Furthermore, the beneficial features of the plant tissue culture technique by providing genetically uniformed disease-free true-to-type plant propagation within a short time, and conserving elite variety plantlets makes this technique an inevitable tool for the rapid production of economically important plants in the 21st century. Therefore, this chapter focuses on the different *in vitro* plant tissue culture techniques applied to regenerate *R. serpentina* plants. In addition, the roles of various physical and chemical factors that could affect the regeneration rate, geographical distribution, bioactive compounds and their bioactivity have also been discussed. The comprehensive data could be helpful for further studies on this valuable plant.

Keywords: Bioactive compounds, Micropropagation, Organogenesis, Pharmacology, *Rauvolfia serpentina*, Somatic Embryogenesis.

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INTRODUCTION

Rauvolfia serpentina (L). Benth. ex Kurz., commonly known as Indian snakewood or Sarpagandha, is considered to be one of the most valuable plants, as it shows a wide range of medicinal properties and contains the world's first antihypertensive drug, reserpine [1]. In literature, like Ayurveda, Siddha, and Unani, it has been found that the roots of *R. serpentina* have been used to cure diseases like high blood pressure, anxiety, insomnia, epilepsy and several central nervous system diseases [2]. *Rauvolfia serpentina* originated in tropical and sub-tropical climatic regions of South-East Asia, and grows up to the elevation of 1300–1400 m. It is indigenous to moist deciduous forests of the Himalayas and Indian peninsula and occurs in India, Bangladesh, Bhutan, Nepal, Pakistan, Sri Lanka, China, Myanmar, Indonesia, Malaysia and Vietnam [3 - 9].

R. serpentina contains an array of bioactive compounds [7, 10]. Among these, the alkaloids are chiefly the reasons for the major bioactivities of *R. serpentina.* The roots and root bark of *R. serpentina* are potent sources of more than 30 indole NAA alkaloids (0.7–2.4%), the most crucial one being reserpine [6, 10, 11]. Reserpine is the most effectively utilized medicinal phytocompound isolated from *R. serpentina* and is a natural tranquilizer. It is a sympathomimetic agent that acts on the sympathetic nervous system, controls cardiac contractions and heart rate, and lowers blood pressure during hypertension. Even in minimal oral doses, reserpine demonstrates its antihypertensive actions by acting as a depressant on the central and peripheral nervous systems [11, 12].

Nevertheless, the overexploitation of this shrub for pharmaceutical utilization has threatened its natural vegetation in some parts of the Southern Western Ghats and North-East regions of India [13]. Besides overexploitation, other issues that caused the decline of *R. serpentina* vegetation at a high rate are its nominal seed germination rate because of poorly viable seeds and a significantly lower rate of vegetative propagation through cuttings [14]. These issues have been marked as severe drawbacks for large-scale production, and eventually, *in vitro* propagation became the solution for these issues [3].

The optimum production of *R. serpentina* can be achieved using several formulations containing various combinations of cytokinin, auxin and additives. *In vitro* propagation of *R. serpentina* has been in attention since the late 20th century [15]. In the 21st century, newly adopted biotechnological approaches made this technique more competent for industrial and conservational purposes [16].

MICROPROPAGATION OF *RAUVOLFIA SERPENTINA*

Factors Affecting Micropropagation

For the *in vitro* regeneration of *R. serpentina* through micropropagation, shoot tips, leaves, nodal and internodal pieces, roots, and embryos have been chosen by several experimenters as explants (Fig. **1**, Table **1**). Many reports on *R. serpentina* suggest that a temperature of 24–25 ± 1–2° C can be the best for plant growth [4, 6]. Besides the temperature, a light intensity of 3000 lux and a 16h photoperiod were found to be optimum for *in vitro* regeneration [10]. The *in vitro* culture experiments have maintained a relative humidity of 50–70% for proper growth of tissues [3]. As the *in vitro* propagation is conducted in a heterotrophic condition, it is evident that the supplemented amount of carbon has played a pivotal role in the *R. serpentina* organ development process. Commonly 3.0% sucrose has been widely used in the case of *R. serpentina* micropropagation. However, some investigators found that half strength of sucrose or a range of 0.03-3.0% sucrose can also be an alternative (Table **1**). In *R. serpentina*, many authors have used MS (Murashige and Skoog, 1962) medium for its relatively high content of nitrate-ammonium salts (Table **2**). Woody plant media, another widely used nutrient medium for woody shrubs, is also used in the *R. serpentina* culture [36].

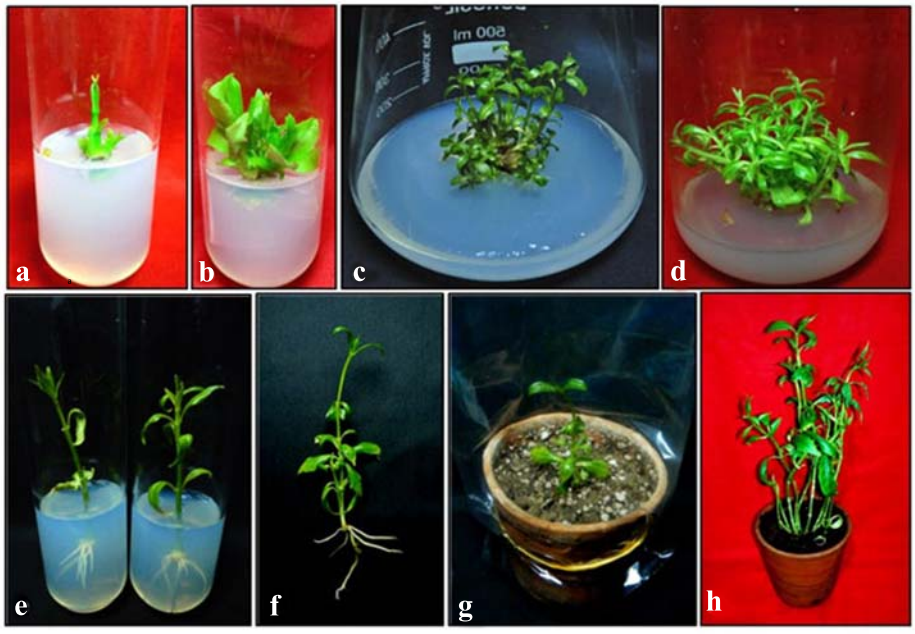

Fig. (1). Micropropagation of Rauvolfia serpentina; (**a–b**) initiation and growth of shoot primordia, (**c–d**) multiplication of shoots, (**e**) *In vitro* rooting, (**f**) a rooted plantlet, (**g**) acclimatization of rooted plantlet, (**h**) *ex vitro* plant.

CHAPTER 14

Micropropagation of *Wrightia* Species

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Abstract: The genus *Wrightia* belongs to the Apocyanaceae family and encompasses 32 species. This genus has many pharmacological properties and is used for many of the human ailments in the traditional systems of medicine. It also has commercial importance for its timber, dye, *etc*. Due to its commercial importance, some of the species of this genus, like *Wrightia tinctoria* and *W. arborea* are overexploited and have become endangered. There is a need to conserve these species. One of the techniques to conserve plants and multiplication is micropropagation. In this chapter, regeneration studies that include collection, sterilization, shoot and root generation, and acclimatization of *Wrightia tinctoria* and *W. arborea* are described.

Keywords: Acclimatization, Auxins, Cytokinins, Explant, *in vitro* Seedling, Microrpopagation, Nodes, Root Regeneration, Shoot Regeneration, Sterilization. *Wrightia*.

INTRODUCTION

The genus of *Wrightia* belongs to the Apocyanaceae family. This genus is distributed throughout the world as shrubs or small trees. It has many pharmacological properties. Dao [1] identified 32 species and out of which a few species of *Wrightia* are *W. tinctoria, W. arborea, W. coccinea, W. mollissima* and *W. pubescens* [2]. Through the literature survey, it was found that micropropagation studies were carried out for four species, *i.e.*, *W. tinctoria, W. arborea* (Syn.: *W. tomentosa*), *W. religiosa* and *W. sirikitae.* In this chapter, micropropagation studies of *W. tinctoria* and *W. Arborea* are reviewed.

MICROPROPOGATION IN *W. TINCTORIA* ROXB.

Wrightia tinctoria Roxb. is a small to medium-sized deciduous tree that produces milky white latex. It is a flowering plant that can be found in Australia, India, My-

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anmar, Nepal, Timor, and Vietnam. It is referred to as the Pala indigo plant or dyer's oleander, Sweet indrajao, Dudhalo, Dudhi, Mitha-indrajau, Karayaja, Kala Kuda and Ankudu chettu. It inhabits both dry and moist regions within its habitat. It has anti-helminthic, antidiarrheal, anti-psoriatic, diuretic, anticancer, antiulcer, analgesic, and antioxidant properties [3].

The herb has historically been used to cure conditions such as psoriasis, eczema, scabies, jaundice, leukaemia, gynaecological diseases, toothache, headaches, dandruff, and diarrhoea. The white soft wood is used for furniture, toys, matchboxes, miniature boxes, turnery, and carving.

W. tinctoria has a short seed viability period and low germination rate and cutting vigour. Due to overexploitation and a lack of quick natural regeneration, the population of this plant has significantly decreased. There is a need for widespread propagation and protection of this precious softwood plant because of these factors, as well as the challenges the toy manufacturing sector faces. One method for the speedy regeneration of propagules that is available and well-established is micropropagation [4]. It provides a quick way to produce clonal planting stock for afforestation, the production of woody biomass, and the preservation of rare and elite genotypes.

As this plant has medicinal and economic value, many scientists like Purohit and Kukda [4, 5], Kairamkonda *et al*. [6], Aftab *et al*. [7], Arulanandam *et al*. [8], Mridula and Nair [9] and Priya *et al*. [10] have worked on the regeneration of this plant using different explants like nodal segments from *in vitro* seedling and adult tree internodes, leaf segments, hypocotyls *etc.*

Materials and Methods

All the required chemicals like mercuric chloride, ethyl alcohol, Tween 20, Labolene, activated charcoal, sucrose, ascorbic acid, agar, bavistin, soilrite, *etc.*, and hormones such as IAA (Indole acetic acid), NAA (Naphthaleneacetic acid), BAP (6-Benzylaminopurine), 2,4-D (2,4-Dichlorophenoxyacetic acid), IBA (Indole butyric acid), Kn (Kinetin), GA (Gibberellic acid), TDZ (Thidiazuron), Phloroglucinol, 2,4,5-T (2,4,5-Trichlorophenoxyacetic acid), *etc.*, were procured from Fischer Scientific, Sigma Aldrich, Thermo Scientific, Karnataka Explosives and High Media, India respectively.

Media Composition

MS medium [12]:MS salts - 100 mg L^{-1} myoinositol, 2 mg L^{-1} thiamine-HCl, 0.5 mg L^{-1} pyridoxine-HCl, 0.5 mg L^{-1} nicotinic acid) containing 3% (w/v) sucrose and supplemented with different concentrations of required auxins or cytokinins.

The pH of the media was adjusted to 5.8 either with 0.1 N NaOH or 0.1 N HCl before adding 0.8% (w/v) agar-agar prior to autoclaving. The medium was sterilized at 121ºC under 15psi in an autoclave for 15-20 min.

Culture Conditions

The cultural conditions for *in vitro* seed germination for the first five days are dark at 72 ^{0} humidity and 25^{0} C temp and later 8 h dark and 16 h light regime.

On the other hand, cultural conditions for shoot and root regeneration are 29±2^{0} C during the day and 25±2^{0} C during the night with 2 photoperiods of 10±2 hours and 12±2 hours and 72% humidity.

Explants, Sterilization, Media, Inoculation and Callus Induction

Purohit and Kukda [5] developed an *in vitro* method for the propagation of *W. tinctoria* using cotyledonary node segments. They used both cotyledonary nodes and hypocotyls from 21 days old *in vitro* seedlings as explants. Among these, 2.0-2.5 cm cotyledonary node segments were found most suitable. They gathered ripe, dry *W. tinctoria* follicles from a superior tree in the Kevda woodland area near Udaipur. The seed surface was sterilised for 5 minutes with 0.2% mercuric chloride, after which the seed was thoroughly washed with sterile distilled water and aseptically inoculated with 0.8% water agar for germination.

Purohit and Kukda [4] described an efficient and reproducible *in vitro* clonal multiplication protocol for *W. tinctoria* adult trees. They obtained *in vitro* multiple shoots from nodal segments of more than 30-year-old trees that have axillary branches. The tree was selected based on quality wood and marked, from which shoots are harvested throughout the year. Sterile distilled water with a few drops of Tween-20 was used to sterilize the explants. Surface sterilised nodal segments measuring 2.5–3 cm long and 0.5–0.8 cm thick were rinsed in sterile distilled water after being exposed to 0.1% (w/v) $HgCl_2$ for five minutes. Explants were inoculated vertically on MS [11], B5 [12], woody plant medium [13], SH [14], and White [15] media.

Kairamkonda *et al*. [6] attempted to multiply *W. tinctoria in vitro* using zygotic embryo cultures. Seeds were removed from the fruit and cleaned for 2 hours under running water. 0.1% (w/v) $HgCl_2$ was used to surface sterilize the seeds for 2–3 minutes, followed by 3–4 rinses in sterile distilled water and soaked for 24 h in sterile distilled water. Zygotic embryos were extracted and inoculated on MS medium (MS salts - 100 mg/L myoinositol, 2 mg/L thiamine-HCl, 0.5 mg/L pyridoxine-HCl, 0.5 mg/L nicotinic acid) containing 3% (w/v) sucrose and supplemented with various concentrations (0.5- 2.5 mg/L) of IAA. All IAA

CHAPTER 15

Decalepis hamiltonii Wight & Arn.: An Overview of its Bioactive Constituents and Conservation Strategies

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Abstract: *Decalepis hamiltonii* Wight & Arn. (Family: Apocynaceae) is a climber native to Southern Peninsular India, commonly called Swallow Root. The plant is used in Ayurveda, Siddha and other traditional systems of medicines as a blood purifier, appetizer, rejuvenator, wound healing agent, *etc*. Apart from this, various other medicinal uses and pharmacological properties created a great demand for this plant that has resulted in destructive harvesting practices in the wild. The plant is generally reproduced through seeds; however, in most of the cases, germination is an intricate process due to its poor seed viability and delayed seed production. Hence, its population has gradually declined due to over-harvesting of medicinally important tuber. International Union of Conservation of Nature (IUCN) declared all the species of *Decalepis* as 'Critically Endangered Globally'. In the present chapter, complete information on traditional uses, phytoconstituents and micropropagation of ethnomedicinally important and critically endangered species *D. hamiltonii* is discussed.

Keywords: Apocynaceae, Critically Endangered, IUCN Red List, Swallow Root, Traditional Medicine.

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INTRODUCTION

Decalepis hamiltonii Wight & Arn. (Family: Apocynaceae) is a climber commonly called Swallow root in English, Maakali beru in Kannada, Nannarikommulu in Telugu and Magalikizhangu in Tamil. The plant is native to Southern Peninsular India and distributed as patches over the rocky slopes and crevices of deciduous forests of Tamil Nadu, Andhra Pradesh, Telangana and Karnataka states [1]. However, its population has gradually declined due to over-harvesting of its medicinally important tuber. The International Union of Conservation of Nature (IUCN) declared all the species of *Decalepis* as Critically Endangered Globally [2].

D. hamiltonii is used in Ayurveda, Siddha and other traditional systems of medicines as a blood purifier, appetizer, rejuvenator, and wound healing agent [3]. The whole plant is used in the treatment of and bronchial asthma, intrinsic haemorrhage, erysipelas and fever [4]. Tuberous roots contain a highly aromatic flavor, which finds its use as an herbal health drink called 'Nannari' prepared by the 'Yanadi' tribe, the Nallamalai forest of Andhra Pradesh [5]. It also increases appetite and provides relief from digestive problems [4, 6]. Tuberous roots are also consumed as pickles, decoction and juices by the tribes of the Western Ghats of India due to their health-promoting properties [3]. Apart from this, various other medicinal uses, pharmacological activities, antimicrobial and food preservative properties [7] created a great demand for this plant, and that has resulted in destructive harvesting practices in the wild. The plant is generally reproduced through seeds; however, in most of the cases, germination is an intricate process due to its poor seed viability, hard seed coat and delayed seed production [8].

PHYTOCONSTITUENTS

Phytochemical studies of *D. hamiltonii* have shown the presence of phenols, flavonoids, tannins, saponins, triterpenes, aldehydes, ketones, sterols, fatty acids, resinol, cardiac glycosides and volatile flavour compounds [4]. Essential oil isolated from hydrodistillation of aromatic tuberous roots yielded 18 compounds, of which 2-hydroxy-4-methoxybenzaldehyde was the major compound with 37.45% composition, followed by 2-hydroxy-benzaldehyde (31.01%), 4-O-methylresorcylaldehyde (9.12%), benzyl alcohol (3.1%), β-caryophyllene (1.19%), and α-atlantone (2.06%) [9]. Similarly, in another study Nagarajan *et al*. [10] reported 2-hydroxy-4-methoxybenzaldehyde as the major compound with 96% composition in essential oil isolated from aromatic roots, followed by vanillin (0.45%), methyl 2-phenylethyl alcohol (0.081%), salicylate (0.044%), salicylaldehyde (0.018%), benzyl alcohol (0.016%), ethyl salicylate (0.038%) and

p-anisaldehyde (0.01%). Reddy and Murthy [11] have also reported 2-hydroxy-4-methoxybenzaldehyde as a major compound with 96% composition in essential oil isolated from the root.

Gas Chromatography and Mass Spectroscopy (GC-MS) analysis of the methanol extract of root showed major compounds such as octadecanoic acid, n-hexadecanoic acid, oleic acid, linoleic acid methyl ester, benzaldehyde, 2-hydroxy-4-methoxy and dodecanoic acid [12]. Selvaraj *et al*. [13] reported 10 major compounds from the methanolic root extracts and the major phytoconstituents were β-D-mannofuranoside, methyl (27.425%), followed by 2-undecene 5-methyl (20.362%); 2-furancarboxaldehyde,5-(hydroxymethyl) (15.711%); benzaldehyde, D-glycero-L-gluco-heptose (8.611%), octadecanoic acid (7.575%); 2-hydroxy-4-methoxy (6.650%) and 4-ethyl-2- hydroxycyclopent-2-en-1-one (5.377%). Mohan *et al*. [14] reported several compounds from the methanolic extract of root, such as furfural, methyl-2-furoate, 2-hydroxy-4-methoxy benzaldehyde, vanillin, tetradecane, diethyl phtalate, hexadecane, carbromal, lupeol, norolean-12-en respectively along with other minor constituents. Giridhar *et al*. [15] raised *in vitro* roots and further extracted with dichloromethane, and dissolved in ethanol solvent. GC-MS analysis revealed the presence of a flavor compound named 2-hydroxy-4-methoxy benzaldehyde with a quantity of 40 ± 2.1 µg/g dry weight extract.

VEGETATIVE AND *IN VITRO* MICROPROPAGATION STUDIES

Seed Germination Studies

The hairy seeds of *D. hamiltonii* collected from Sathyamangalam forest area of Tamil Nadu state, India subjected to germination studies [16]. A germination test was conducted in four replications of 100 seeds with three different pre-treatments (24 hours soaking in normal water, hot water and 1000 ppm gibberllic acid) and allowed to germinate using sand and filter paper. Results revealed that seeds soaked in hot water (60°C) for 24 h on moist filter paper as a substrate found to be the most significant method for germination with the highest germination percentage of 83 to 98%. Pretreatment study coupled with microscopic observations revealed that 14% of seeds were hard coated, and they could tolerate the desiccation level up to 10% moisture. The total mortality rate of seeds was at 10°C, and rapid depletion of seed metabolites was reported after 4 months of storage. The short viability of seeds and hard seed coats, coupled with the innate germination process, hinder the plant from the natural regeneration process [16].

CHAPTER 16

Micropropagation of Pharmaceutically Important Plant *Tinospora cordifolia* (Willd.) Hook. f. & Thomson: An Overview

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Abstract: *Tinospora cordifolia* (Willd.) Hook. f. & Thomson belongs to the family Menispermaceae. The origin of the species is from Indian subcontinent to Indo-China. The plant has antipyretic, antiperiodic, anti-inflammatory, antirheumatic, spasmolytic, hypoglycaemic and hepatoprotective properties. Due to over-exploitation of this medicinally important species, attention has been given to its conservation through *in-vitro* micropropagation techniques. The present chapter emphasizes the ample research work on *in-vitro* regeneration and enhancement of secondary metabolites from *T. cordifolia*. Additional information on the importance of this genus in various systems of medicine, active components, biological activities and its sustainable utilization for the welfare of mankind is also discussed in the present chapter.

Keywords: Conservation, Callus Culture, *In vitro* Regeneration, Micropropagation, Menispermaceae, Traditional Medicine.

INTRODUCTION

Plants are not only the manufacturers of food but also produce various types of alkaloids, terpenoids, saponins, phenols and many other secondary metabolites, which are often used as medicines either in raw or processed form. Interestingly,

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nearly one-quarter of modern medicines comprise of plant sources, such as herbal extracts [1]. Due to the over-exploitation, the medicinal plants are under enormous threat of declining population size, genetic diversity and loss of habitat [2]. Demand for active constituents of plant origin in several pharmaceuticals, fragrances, flavors and color industries exceeded several billion dollars per year [3]. In this scenario, the *in vitro* plant tissue culture method for propagation and also as a source of producing important bioactive secondary metabolites paves the way for conservation and mass multiplication of important medicinal plants. In recent years, plant tissue culture has become of major industrial importance not only in the area of propagation but also in the production of quality materials and large-scale natural metabolites. In this non-conventional method of propagation, thousands of plantlets can be produced from a small piece of tissue (explants) in a relatively short duration under controlled conditions, irrespective of season and weather. Hence, it is also called a low cost-high volume system for the production of biomass and several bioactives present in it. Similarly, as the commercial values for plant secondary metabolites are evolving, eventually, tissue culture technique plays a major role in providing a continuous supply of healthy plant materials to several pharmaceutical industries. Therefore, rapid regeneration and production of high quality, uniform planting material through reliable micropropagation methods are important for the economically important medicinal plants are the needs of the time [4].

Tinospora cordifolia (Willd.) Hook.f. & Thomson belongs to the family Menispermaceae. The origin of the species is from the Indian subcontinent to Indo-China and distributed over East Himalaya, Myanmar, Bangladesh, India, Maldives, Sri Lanka and Vietnam [5]. It is generally a deciduous woody climber or climbing shrub and can also grow from its detached stem [6].

In India, *T. cordifolia* is described in the ancient Ayurvedic texts like *Charaka Samhita, Sushruta Samhita* and also in *The Ayurvedic Pharmacopoeia of India*. *T. cordifolia* is used to treat diabetes, gastrointestinal disorders, dyspepsia, flatulence, gastritis, jaundice, diarrhea and hemorrhoids [7]. The plant has antipyretic, antiperiodic, anti-inflammatory, antirheumatic, spasmolytic, hypoglycaemic and hepatoprotective properties. The whole plant extract increases the urine output, and the stem juice is used to treat fever. The decoction of the whole plant is used in rheumatic pain, bilious fever and as a febrifuge. The starch extracted from the stem has anti-diarrhoeal, antidysenteric and antacid properties [7 - 9]. Plant stem acts as a diuretic, stimulates bile secretion, enriches the blood, helps in relieving constipation, vomiting, burning sensation, cures jaundice, and a mixture of stem and root juice is used as an antidote for snake bite and scorpion sting [9]. It has known to possess immunomodulatory, anti-diabetic, anti-toxic, anti-arthritic, anti-osteoporotic, anti-HIV, anti-cancer, anti-microbial, anti-

oxidant, anti-tumor, anti-hypoglycemic, anti-dipressant, cardioprotective, anti-ulcer, anti-diarrheal, analgesic, aphrodisiac, neuroprotective, anti-inflammatory, gastroprotective, radio protective, hepatoprotective, antipsychotric, anti-asthmatic anti-malarial, anti-allergic, larvicidal and anti-severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) properties [10 - 18]. Active constituents such as berbarine, boldine, choline, clerodane derivatives, columbamine, columbin, cordifolide A, cordifolide B, cordifolide C, cordifolioside A, cordifolioside B, cordifolioside C, cordifoliside D, cordifoliside E, cordioside, cordioside, ecdysterone, furanoid diterpene glucoside, giloinsterol, furanolactone and several other compounds have been reported from *T. cordifolia* [10, 12 - 15, 19 - 23].

IMPORTANCE OF PROPAGATION METHODS

It is reported that *T. cordifolia* is one of the key ingredients used in about 69 Ayurvedic formulations, and it has a huge demand in local as well as international markets due to its wide therapeutic values. Since the demand for raw materials of *T. cordifolia* has increased from 2000 to 5000 MT with an annual growth of 9.1%, the National Medicinal Plant Board (NMPB), India has prioritized this species for its mass multiplication program throughout the country [24].

Even though it can grow by its detached stem, there are a few reports on vegetative propagation [6]. Vegetative propagation through stem cuttings with four lateral buds had significantly better survival rates than the stem cuttings with one to three buds. However, single bud stem cutting proved to be economical for one hector plantation compared to available conventional methods, even though the survival depends on its morphological traits and age of the stem cutting. In the traditional propagation method (stem cuttings with single lateral nodes and planted in a horizontal position as in sugarcane), it consumes a large space in the nursery [25].

IN-VITRO STUDIES

T. cordifolia species may be propagated through stem cuttings; however, there is little information available about the propagation through seeds. The stem part may have the rejuvenating capacity and can even be grown by vegetative multiplication. However, currently, there is a need to grow the plant along with enhancing its active constituents for pharmaceutical industries. Because of the increasing demand due to its medicinal values, it is important to conserve these species and, at the same time, the demand for its medicinal purpose to be met. Partially, it is being fulfilled by agro-industries by growing them in the field through stem cuttings. However, the enhancement of secondary metabolites through callus culture or hairy root culture techniques seems to be the more suitable option for scaling up the required biologically active components, as it

CHAPTER 17

A Systematic Review of Phytoconstituents and Tissue Culture Studies of the genus *Hoya* R. Br.

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Abstract: The genus *Hoya* (Family: Apocynaceae) has more than 500 species, comprising mainly of epiphytes and geographically distributed in South America, Southeast Asia, Indo-Malesia and Australian regions. Most of the species are cultivated for their ornamental, aromatic and showy flowers. Philippines is one of the countries with the highest diversity of *Hoya* species. As seed setting is very rare in most of the species, it necessitated the development of conservation strategies through *ex situ* conservation methods using vegetative or micropropagation techniques. Present chapter provides detailed information on the traditional uses, phytoconstituents, conservation status and micropropagation studies of the ornamental and medicinally important genus *Hoya*.

Keywords: *Hoya*, Endangered, Conservation, Bioactives, Ornamental Plants, Horticulture.

INTRODUCTION

The genus *Hoya* R. Br. with more than 500 species, is one of the largest genera in the family Apocynaceae [1]. The species are mainly epiphytic lianas, often twining or climbing by adventitious roots and known as wax plants due to the appearance of their leaves and flowers. Of all, more than 300 species of *Hoya* are

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distributed in tropical and sub-tropical Asia to West Pacific [1, 2]. *H. imperialis* and *H. coronaria* are prevalently cultivated for their beautiful, aromatic and ornamental flowers in Europe, America and Australia. However, the propagating methods of these plants have not been reported yet. Wild populations of *Hoya* in Brunei Darussalam have become highly threatened because of habitat loss and overexploitation [3].

The Philippines Island is considered one of the eight hottest biodiversity hotspots in the world and has the highest proportion of endemic and threatened vascular plants [4, 5]. Moreover, it is one of the countries with the highest diversity of *Hoya* species, and reported from all the altitudes of the Archipelago at different types of habitats such as limestone cliffs, boulders and swamp forests. More than hundred species of *Hoya* have been recorded throughout the country. In Philippines, Mindoro is the 7th largest island, and interestingly, 18 *Hoya* species have been reported from this island alone [6]. Among these, 15 species are endemic to Philippines. It is also a notable fact that among these 18 species, three are included in the endangered category, such as *Hoya alagensis* Kloppenb., *Hoya halconensis* Kloppenb. and *Hoya paziae* Kloppenb.

Indonesia is another country which has a rich diversity of *Hoya* species. In Ketori forest areas of Indonesia, 14 *Hoya* species were identified, and most of them have ethnomedicinal importance as reported by several indigenous tribes. Further, some of the *Hoya* species were found to be used as vegetables and medicines as well. It was found that the people communities of Ketori forest frequently use *H. coronaria* and *H. meredithii* as raw vegetables and they believe that these species have the ability to decrease high blood pressure. Another species *H. waymaniae* was used to treat stomach and headache. It is an interesting fact that the people of Ketori village practice their own local traditional rules for sustainable utilization of forest products and management of local biodiversity [7].

A compound isolated from *H. multiflora* used to treat several diseases including rheumatistm, abdominal pain, asthma and intestinal inflammations [3]. Leaf extract of *Hoya parasitica* Wall. used to treat fever, body pain, rheumatism, kidney problems, urinary tract disorders and jaundice [8, 9]. Leaf paste of *Hoya globulosa* Hook. f. used in the treatment of bone fractures [10]. Crushed leaves of *Hoya coronaria* Blume. are used to cure cut wounds, and leaf paste of *Hoya potsii* Trail found effective in the treatment of injury, gynecological disorders, rheumatoid arthritis and digestive disorders [11]. *Hoya kerrii* has long been used as a folk medicines for curing inflammatory-related disorders [12]. Cold macerated extract of *Hoya vanuatensis* green young leaves used as an oxytocic agent [6, 13, 14].

PHYTOCONSTITUENTS IN *HOYA*

The attractive flower shapes and pleasant fragrance of some *Hoya* species make them suitable for a new source of aroma. Basir *et al*. [1] studied the biosynthesis of secondary metabolites and volatile fragrances from three *Hoya* species such as *H. cagayanensis* C.M.Burton, *H. lacunosa* Blume and *H. coriacea* Blume, using solid phase micro extraction. Further, gas chromatography mass spectroscopy (GC-MS) analysis, phytochemicals and transcriptomic methods were explored to elucidate the mechanism of perfume synthesis, particularly terpenoids. GC-MS analysis revealed the presence of 23, 14 and 36 compounds in *H. cagayanensis*, *H. lacunosa*, and *H. coriacea*, respectively. Volatile components showed different fragrance profiles from all three *Hoya* species. Monoterpene compounds such as β-ocimene (25.75%) and methyl salicylate (24.67%) were dominated in *H. cagayanensis*, whereas the alcohol component 1-octen-3-ol (26.1%) and the ester compound named (Z)-butyric acid, 3-hexenyl ester (29.36%) were the major compounds in *H. lacunosa* and *H. coriacea*, respectively.

Ebajo Jr *et al*. [15] isolated total seven compounds from dichloromethane extracts of an endemic Philippine ornamental plant, *H. buotii* Kloppenb. Compounds taraxerone, taraxerol, and a mixture of β-sitosterol and stigmasterol were isolated from roots and stems. The mixture of α-amyrin cinnamate and β-amyrin cinnamate was isolated from flowers. At the same time, squalene was also reported from leaves. Ragasa *et al*. [16] isolated number of bioactive compounds from the dichloromethane extracts of *H. cumingiana* Decne. The extract afforded α-amyrin, β-amyrin, bauerenol, lupeol, β-sitosterol, stigmasterol and taraxerol from different parts of the plant. Similarly, dichloromethane extract of stem and leaves of *H. cagayanensis* yielded dihydrocanaric acid, lupeol, lupenone, 2-hydroxyethyl benzoate, β-sitosterol and stigmasterol [17].

VEGETATIVE AND *IN VITRO* MICROPROPAGATION STUDIES

In vitro micropropagation of *H. wightii* ssp. *palniensis*, a highly vulnerable and endemic species of the Western Ghats, Tamil Nadu, India was reported by Revathi Lakshmi *et al.* [18]. Shoot tip explants were cultured on Murashige and Skoog medium (MS medium) containing different concentrations and combinations of cytokinins [Thidiazuron (TDZ), 6-(γ,γ-Dimethylally- lamino)p urine (2-iP), Kinetin (Kn) and Benzyl adenine (BA)] and auxins [Naphthalene acetic acid (NAA), Indolebutyric acid (IBA) and Indole-3-acetic acid (IAA)]. High frequency of shoot proliferation and multiple shoot induction was observed in the media containing Kn (4.65 μM) + IBA (1.47 μM), supplied with 100 mg/L ascorbic acid. Rhizogenesis was observed on MS medium supplemented with IBA

CHAPTER 18

In vitro Regeneration and Conservation of the Medicinal and Aromatic genus *Kaempferia*: An Overview

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Abstract: Genus *Kaempferia* comprises about 124 species distributed in Southeast Asia and is well known for it's diverse medicinal, nutritional and industrial values. The plants of the genus are rhizomatous, perennial, and oil-yielding plants; some are also used as spices. The essential oil obtained from the plants has a considerable market value worldwide. The rhizomes of these plants were used in traditional medicine due to the presence of diverse bioactive compounds and used to treat urinary tract infections, fever, cough, hypertension, metabolic disorder, asthma, rheumatism, epilepsy, skin diseases, *etc*. Seed dormancy, seasonal outgrowth and seed made through cross-pollination were found to be non-viable, which are the prime limitations of *ex situ* conservation regarding this genus. To overcome this type of problem, *in vitro* tissue culture is the way to get the plants available over the year without any limitations. This chapter is based mainly on exploring those bioactive compounds containing species of the genus *Kaempferia*, and obtaining an alternative resource of phyto-compounds for use in pharmaceuticals and conserving them through an artificial way to get them throughout the year without exploiting the area and genotypic alteration.

Keywords: Conservation, *Kaempferia*, Micropropagation, Phyto-compounds, Zingiberaceae.

INTRODUCTION

Zingiberaceae is one of the largest and most exploitable plant families, comprising 53 genera and more than 1300 species worldwide and distributed chiefly in tropical and subtropical areas. The plants under this family are perennial, rhizomatous, oil-yielding medicinal herbs used worldwide as spices, food, medicine, and others. Due to the enrichment of essential oil with diverse compounds, this family's plants also have significant aromatic, medicinal, nutriti-

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onal, and ornamental properties. The genus *Kaempferia* consists of more than 100 species (The World Flora Online, Plants of the World Online, The Plant List) and is distributed throughout Southeast Asia. Species included under this respective genus are *Kaempferia rotunda, K. galanga, K. parviflora, K. angustifolia,K. marginata, K. pandurata, etc*., explored with a diverse range of bio-activity. The plant species are well known for treating allergies, arthritis, cancer, cardiovascular disorders, inflammation, sexual dysfunction, skin integrity, *etc* [1]. *K. parviflora* is used as a food supplement for treating metabolic disorders in Japan [2]. Rhizomes of *K. galanga* are used as a spice in cooking worldwide and sold as an industrial crop in the market [3]. *K. elegans* is well known for its medicinal uses, also known as an ornamental plant in Vietnam [4]. Other species belonging to the genus are also known for their utilization as food, medicine, spice, and many more. Hence, all the species explored, not or adequately, should have some activities that can be used as an alternative resource in different branches of medicine development.

The plants of the family Zingiberaceae are seasonal and found at a specific period in the environment. This is also a limitation of this plant species, that we cannot obtain them throughout the year. Conservation is the only alternative path that should be followed to prevent the plants from vanishing and to obtain them throughout the year from the environment. The massive depletion of medicinal plants for a continuous supply of phytocompounds is becoming a prime threat to their extinction from their natural habitat. Large-scale *in vitro* production of plants is a useful alternative to maintain Phyto-diversity from depletion in the natural environment. *In vitro* conservation of the species of *Kaempferia* is the safest way to protect the elite germplasm from extinction due to disease, natural disaster, or extensive non-scientific use. As the plants belonging to this genus are seasonal, and outgrowth is visible in a respective season in the year, *in vitro* tissue culture must help to obtain the plant over the year for a continuous supply of phytocompounds.

Medicinal Importance of the Genus *Kaempferia*

The species belonging to the genus *Kaempferia* are well-known for their medicinal uses. Several *Kaempferia* species, like *Kaempferia galanga, Kaempferia parviflora, Kaempferia angustifolia, Kaempferia rotunda*, and *Kaempferia elegans,* are used in folk medicine, mainly in southeast Asia from the ancient era. However, few are used to develop modern medicine under research to combat emerging diseases.

Kaempferia galanga is one of the most well-explored species belonging to the genus *Kaempferia* with diverse medicinal importance. The rhizome of this plant is

well-known for essential aromatic oil, comprised of many bioactive compounds with high therapeutic index. *K. galanga* is also well known for its aromatic nature; hence, it is often used as a spice. The nutritive value of this rhizomatous plant is also high and taken as food in different countries. Essential and non-essential minerals and ions like calcium, potassium, manganese, and chromium are also present in the rhizome, which is used enormously. The plant is used for flatulence, laxative, stomachache, tonic, intoxication, antiangiogenic, sedative, diuretic and vasorelaxation activity [5 - 11]. The antimicrobial, antioxidant and cytotoxic activity of this plant has been tested previously against many pathogenic as well as non-pathogenic organisms by different researchers [12 - 14]. The plant is proven to be an excellent antimicrobial agent and causes the elimination of different multi-drug resistant microorganisms. Anti-tumour and anticancer activity of several compounds and the crude extract of *K. galanga* have also been reported earlier [15 - 17].

Another important species is *Kaempferia parviflora* has significant medicinal importance. The rhizome of this plant is well-known for antibacterial, anti-obesity, anti-diabetic, cardiovascular protective, immunoregulatory, neuroprotective, and skin-whitening activity [1, 18 - 20]. The rhizome extract also reduces visceral fat in overweight Japanese adults [21]. Anticancer activity of this plant on different cell lines has also been reported [22]. *K. parviflora* was also reported to enhance sexual performance in traditional use [23, 24]. Furthermore, the compounds polymethoxyflavones have an anti-ageing ability and are used in cosmetics and nutraceutical products [25]. The plant extract is also used for wine fermentation as a base composition [26].

Kaempferia rotunda is another plant species with widespread applications, including treating indigestion, fever and wound healing properties [27]. The plant has been reported for its anticancer activity against different cancer cell lines. *K. rotunda* can prevent the proliferation of colon cancer cells SW480 and SW48 by causing apoptosis in the intrinsic mitochondrial pathway [28]. Strong anticancer activity against breast cancer and pancreatic cancer of different solvent extracts of this plant has been reported by several researchers in the past [29, 30]. Antioxidant, anti-proliferative, anti-inflammatory and antibacterial activity of rhizome extract has been reported for this plant [31 - 33]. Silver nanoparticle synthesis from the plant rhizome and their activities towards tumour growth suppression in mice was reported [32]. This is an essential aromatic oil-yielding plant; the oil is a food preservative, flavouring, and antibacterial agent in different aspects [34]. The plant is also well known for its nematicidal and immunomodulatory activity [35, 36].

CHAPTER 19

Micropropagation of *Stemona tuberosa* Lour. –A Review

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Abstract: The present review summarises the *in vitro* multiple plantlet regeneration of *Stemona tuberosa*. MS medium fortified with 7 mg/L Kn was found to be the optimum for multiple shoot induction from axillary buds. Excision and culture of nodal segments from the *in vitro* shoots on medium containing 7 mg/L Kn and 4 mg/L TDZ showed a maximum number of shoot multiplication. Shoots developed were rooted best on ½ strength MS with 1 mg/L IAA. Plantlets established in pots exhibited 85% survival.

Keywords: Micropropagation, Multiple Shoots, *Stemona tuberosa*.

INTRODUCTION

Stemona tuberose Lour., belonging to the family Stemonaceae, is mainly distributed in India, Bangladesh, Nepal, Myanmar, Cambodia, Vietnam, Thailand, Taiwan and China. It is a twiner with tuberous rhizome, leaves ovate with basal nerves, flowers bisexual, axillary, anther dorsifixed, petal-like connective long, fruit ovoid-oblong capsule, and 5-8-seeded (Figs. **1A** & **B**).

The tuberous roots of *S. tuberosa* have antibacterial, antiparasitic and expectorant properties. They are used in the treatment of coughs, ascariasis and oxyuriasis. The tuberous roots show bacteriostatic activity and are used in phthisis and cough. The drug soothes the respiratory centers without affecting the heart [1].

Herbal extracts from the species of *Stemona* have been used for treating of respiratory diseases and as anthelmintics in Asian countries for thousands of years [2, 3]. *Stemona tuberosa* has been officially listed in the 2005 edition of the Chinese Pharmacopoeia as antitussive traditional Chinese medicinal herb [4]. In

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India, various *Stemona* species are used as insecticides and traditional medicines, *e.g*., treatment of skin diseases, killing head lice and scabicide [5, 6]. The plant is used to cure different human diseases, *viz*., whooping cough, chronic bronchitis, dermatitis, eczema, urticaria, amoebic dysentery, psoriasis, trichomonas vaginitis, pinworm disease, and pulmonary tuberculosis [7, 8]. *S. tuberosa* has two alkaloids, neostenine and neotuberoStemonine, which showed antitussive activities [9].

Four new dehydrotocopherols (chromenols) have been isolated from different species by Brem *et al.* [10]. Based on TLC tests and microplate assays with the free radical DPPH, the antioxidant capacities of all chromenol derivatives were comparable with that of α-tocopherol.

The Stemonaceae is so far the only source of the *Stemona* alkaloids. Pilli *et al.* [3] discussed the biological activity and natural sources of *Stemona* alkaloids. The biological activities of some *Stemona* alkaloids have been evaluated in order to find the active principles of *Stemona* species. Tubero Stemonine is the first *Stemona* alkaloid to have its biological activity tested. The anthelminthic activity of this alkaloid was detected against the motility of some helminthic worms, such as *Angiostrongylus cantonensis, Dipylidium caninun*, and *Fasciola hepatica.* TuberoStemonine has been reported as an effective insecticide equivalent with azadirachtin when tested against the larva of *Spodoptera littoralis*. The action of tuberoStemonine on the neuromuscular transmission in crayfish was also investigated, revealing that this alkaloid depressed glutamate-induced responses at similar concentrations to those of established glutamate inhibitors.

Five new Stemoninine-type alkaloids, bisdehydroStemoninine (1), isobisdehydroStemoninine (2), bisdehydroneoStemoninine (3), and bisde- hydro Stemoninines A (4) and B (5), have been isolated by Lin *et al.* [11] from the crude-alkaloid extract of the roots of *S. tuberosa*. Alkaloid 1 displayed significant antitussive activity in the citric acid-induced guinea pig cough model.

Three new croomine-type *Stemona alkaloids,* along with ten known constituents, were isolated by Lin *et al.* [12] from the roots of *S. tuberosa*. The antitussive activity of the major alkaloids was tested using the citric acid-induced guinea pig cough model. Croomine (**8**) exhibited a dose-dependent inhibition of coughing with an ID_{50} value of 0.18 mmol/kg.

Lin *et al.* [13] isolated alkaloids from the roots of *S. tuberosa*, which include stemoenonine (1), 9a-O-methylstemoenonine (2), oxystemoenonine (3), 1,9a-seco-stemoenonine (4), and oxyStemoninine (5), Stemoninoamide (6) and Stemoninine (7). Compounds 6 and 7 exhibited strong antitussive activity after oral and intraperitoneal administrations.

Twelve dihydrostilbenes, stilbostemins N-Y (1-12), and a phenanthraquinone, stemanthraquinone (13), were isolated from roots of *S. tuberosa*, along with five known dihydrostilbenes by Lin *et al.* [14]. Dihydrostilbene 8 exhibited strong activity against *Bacillus pumilus* (MIT 12.5-25 microg/mL). Many tested compounds exhibited moderate antibacterial activities. *In silico* analysis of compounds from *S. tuberosa* inhibited N1 neuraminidase of H5N1 avian virus [15].

IN VITRO PROPAGATION

The main source of plant material is from natural habitat. This plant material is insufficient to meet the growing demands. Moreover, it leads to the disappearance of the plant species in the natural habitat. Over exploitation, habitat destruction, pollination limitations, loss of potential dispersers, scattered distribution and reproduction inability are the main causes for decreasing natural populations [16]. Hence, in a short span of time, it is going to be endangered and later extinct. It is an urgent need to multiply this endangered medicinal plant both *in vivo* and *in vitro*. Field cultivation is unsuccessful because of time consumption. *Ex-situ* conservation efforts have a limited impact on halting the decline in the population. Hence, *in vitro* methods are the only alternative and effective method to produce this plant on a large scale to meet the growing demand globally. Fresh rhizomes can be collected from the wild and used as a propagating material for either *in vivo* or *in vitro* propagation. *In vitro* propagation is a more effective means than *in vivo* methods.

In vitro regeneration is a competent mean of *ex-situ* conservation of plant diversity by using minimum plant material without disturbing the wild habitat. Moreover, *in vitro* propagation can overcome the problems of seasonal variation, reproductive inefficiency, self-incompatibility and susceptibility to diseases.

Stemona spp. can be vegetatively propagated by planting tuberous roots with attached buds; however, this process takes time. Moreover, sexual propagation by seeds is very poor [17]. Therefore, a number of micropropagation protocols have been developed by different researchers in *Stemona* spp., which includes *S. japonica* [18], *S. collinsae* [19], *S. curtisii* [20], *S. tuberosa* [21 - 23], *Stemona* sp [24], and *S. hutanguriana* [25].

Murthy *et al.* [23] collected the live plants of *S. tuberosa* from the college botanical garden for explants source. Nodal explants are considered the best explants for shoot multiplication experiments as they have pre-existing axillary buds. The nodes were excised and washed in the running tap water, followed by a fungicide and bactericide, each 0.3% for 10 min and with Tween 20 (5% v/v for 4 min, Loba Chemie Pvt. Ltd, Mumbai). Then, explants were treated with surface

CHAPTER 20

In vitro propagation of *Oxalis corniculata* L.

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Abstract: The Oxalidaceae family is known for small herbs, shrubs and small trees with economic and medicinal properties in folklore medicines. The genus *Oxalis* is distributed worldwide and is famous for tuberous and ornamental cultivars. The present study established reproducible *in vitro* protocols for mass multiplication of *Oxalis corniculata* L. *via*. micropropagation and indirect organogenesis using different explants. Murashige and Skoog (MS) medium augmented with various cytokinins, auxins and gibberellic acid and combinations with respect to the different protocols. In micropropagation, shoot tip and node explants cultured on a medium with 6-benzyl adenine (BA) 3.0 mg/L, 6-furfuryladenine (Kn) 1.0 mg/L and naphthalene acetic acid (NAA) 0.5 mg/L produced the highest average of 35.1 and 28.5 shoots after 25 days of culture, respectively. Gibberellic acid (GA_3) treatment was satisfactory in shoot elongation, and rooting of shoots was best on indole-3-butyric acid (IBA) 3.0 mg/L than indole-3-acetic acid (IAA) and NAA. In indirect organogenesis, internode, leaf and petiole explants produced green, compact nodular calli at varying frequencies on medium fortified with auxins. The maximum frequencies of shoot regeneration and shoot numbers were observed on a medium containing BA 1.0 mg/L and IBA 0.5 mg/L. Further, the shoot elongation was achieved with BA and GA_3, and rooting was best achieved on IBA 3.0 mg/L with Kn 0.5 mg/L. All the plantlets were successfully hardened and acclimatized under the greenhouse condition with maximum survival of 95%. The current protocols established *via* meristem and callus mediated cultures would help in bioprospecting of this less explored medicinal plant.

Keywords: Callus, Micropropagation, Medicinal Plant, MS Medium, Organogenesis, *Oxalis corniculata*, Rooting, Shoot Elongation, Shoot Tip.

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INTRODUCTION

Plant-based remedies often have minimal unintended effects and are relatively cost-effective. Over 40% of medicines now prescribed in the world contain chemicals derived only from plants, and therefore, demand for traditional herbal drugs is increasing rapidly. At this time of increased demand for high-value medicinal plants, an alternative system is necessitated to protect the natural medicinal plant resources.

The family Oxalidaceae consists of 6 genera and about 770 species with herbs, shrubs, or small trees [1, 2]. The genus *Oxalis* consists of about 500 species worldwide and is known for tuberous plants and ornamental cultivars [3]. *Oxalis corniculata* L. is one among the species, distributed throughout India, South Africa and tropical and sub tropical America. It is commonly known as Indian sorrel in English; Puliyarai in Siddha and Tamil; Chaangeri, Amlapatrikaa, Amlikaa, Chukraa, Chukrikaa, Chhatraamlikaa in Ayurveda; Ambutaa bhaaji, Amutaa saag in Unani; and Tinpatiyaa and Ambilonaa in folk medicine [4].

The leaves of the plant are consumed as raw and cooked food, and the whole plant is used to cure dyspepsia, piles, anemia, tympanites, fever, dysentery, scurvy, snake bite, scorpion sting, and skin diseases [5]. The plant contains major phytochemicals such as carbohydrates, tannins, flavonoids, polyphenols, steroids, alkaloids, volatile oil, fatty acid and glycosides, and several other chemical constituents including tartaric acid, citric acid, oxalic acid, cinnamic acid, malic acid, c-glycosyl flavonoids, ascorbic (I), dehydroascorbic (II), pyruvic, glyoxalic acids, *etc*., and possesses diverse pharmacological properties including antioxidant, anti-cancer, anti-inflammatory, antimicrobial, diuretic, febrifuge, cardio-relaxant, *etc* [6].

In recent times, biotechnological advancement offers attractive opportunities for the production of desired plants and medicinal products using *in vitro* culture systems such as callus cultures, cell suspension cultures and organ cultures, and genetic manipulation. Pieces of literature on *in vitro* propagation of Oxalidaceae members are infrequent and limited among the genera. The present study aimed to establish reproducible protocols *via*. meristem culture and indirect organogenesis using various explants.

MATERIALS AND METHODS

Source of Plant Material

Oxalis corniculata plants were collected from the natural habitats at Kolli Hills, Tamil Nadu, India and maintained in the garden of the Department of Botany,

Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. Plants were collected at different periods from July to September, May to June and December to February and maintained in the garden.

Explant Sterilization

The shoot segments were procured from plants maintained in the garden and cut into small pieces of about 5-7 cm. The excised shoot segments were initially washed in running tap water with 1-2 drops of Teepol (liquid detergent) (Reckitt Benckiser (India) Pvt. Ltd., Gurugram, India) for 5 min. Then, the shoot segments were disinfected with 70% ethanol for 30 s, followed by surface sterilization in 0.1% (w/v) aqueous solution of mercuric chloride ($HgCl_2$) for 5 min, and rinsed with sterile distilled water at least 4 times.

From the sterilized shoot segments, nodes and shoot tips were excised to 0.5-1.0 cm long and cultured upright into the shoot induction medium for micropropagation. Internode, leaf and petiole explants were prepared to 0.5 cm segments and cultured on a callus induction medium by the abaxial surfaces of the leaf, petiole and internodes exposed to the media.

Basal Medium

Murashige and Skoog (MS) medium [7] fortified with 3% sucrose and 0.8% agar (Type I) was used as the basal medium. The pH of the medium was adjusted to 5.7±0.2 before being solidified with agar. About 10-15 ml aliquots of the media were dispensed into the culture tubes and plugged with non-absorbent cotton plugs before being autoclaved at 1.06 kg/cm^2 and 121°C for 15 min.

All the chemicals and media used in the study were purchased from HiMedia®, Bengaluru, India.

Culture Conditions

All cultures incubated under the culture room maintained at 25±2°C, provided with 16-h photoperiod and 35 µM m^{-2} S^{-1} light intensity supplied by cool white fluorescent lamps (Philips, Mumbai, India), and 55-60% relative humidity.

Shoot Bud Induction and Multiplication

The sterilized node and shoot tip explants were cultured on the basal medium containing different plant growth regulators (PGRs). Cytokinins, 6-benzyl adenine (BA) and 6-furfuryladenine (Kn) at 0.5-5.0 mg/L were used either individually or in combination for shoot bud induction. Then, the shoot buds were subcultured on shoot multiplication medium containing BA (3.0 mg/L) and Kn (1.0 mg/L) supp-

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An Effective Micropropagation Strategy for *Pseudarthria viscida* (L.) Wight & Arn.

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Abstract: Habitat destruction and over-harvesting have resulted in the gradual disappearance of many medicinally important plants from their natural habitat. At present, their number is highly reduced in the wild. To conserve the genetic stocks of such plants, *in vitro* propagation can be utilized successfully. One such medicinally important plant that needs to be conserved is *Pseudarthria viscida* (L.) Wight & Arn. It is a perennial viscid pubescent semi-erect, diffuse undershrub belonging to the family Fabaceae. It is an essential component of many famous Ayurvedic formulations like Dashamoola, Mahanarayana taila, and Dhantara taila. The root is the most important part of the plant with high medicinal value. Major chemical compounds reported to be present in the roots are 1,5 dicaffeoyl quinic acid, oleic acid, tetradecanoic acid, rutin, quercetin, gallic acid, ferulic acid, and caffeic acid. The present study focused on *in vitro* regeneration and mass propagation of *P. viscida*. Fresh young leaves, nodes, and internodal segments were used as explants. Murashige and Skoog medium (MS medium), Gamborg's (B_5) medium, and White's mediums were selected for *in vitro* regeneration and mass propagation. Among the various media used, the MS medium gave a successful result in *in vitro* culture by showing a response within four weeks, and the percentage of response was also high compared to B_5 and White's medium. The leafy explant was found to be more suitable for profuse callus induction, somatic embryogenesis, and indirect organogenesis than that of internodal and nodal explants, whereas nodal explant was best for direct organogenesis in *P. viscida.* Of the different combinations tried, NAA (Naphthalene acetic acid) + BAP (6-Benzyl aminopurine) combinations were best for callus induction, somatic embryogenesis and indirect organogenesis. 2.5 mg/L BAP was best for shoot induction from nodal explants, whereas 2.5 mg/L NAA was best for root induction from *in vitro* regenerated micro shoots as explants. Well-developed plantlets were transferred to greenhouse and later to natural conditions. This study thus reports an efficient protocol for plant regeneration, and this could be vital for the multiplication and field transfer of this ethnomedicinal plant. Based on the ethnomedicinal potential, there is an urgent need for organized cultivation of this vulnerable plant for its conservation and sustainable utilization.

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Keywords: Conservation, Dashamoola, Dhantara taila, Ethnomedicinal, Hormonal Combinations, *In vitro* Regeneration, Mass Propagation, Mahanarayana Taila, *Pseudarthria viscida*, Sustainable Utilization.

INTRODUCTION

Medicinal plants have been emerging as a part of the modern life of man and with the greatest demand due to their nutritional, pharmaceutical, cosmetic and medicinal application without much negative impact. Hundreds of medicinal plants are at high risk of extinction due to over-exploitation and habitat destruction. This threatens the invention of future heals for diseases. For primary health care, about five billion people depend on traditional phytoremedies [1]. About 50% of prescription drugs are obtained from phytoconstituents, which were first identified in plants. Commercial and scientific attention to medicinal plants leads to high pressure on them, and continuous harvesting leads to threat and is facing a high risk of extinction. Scientists say that a minimum of one potentially important drug is lost by Earth every two years [2]. Each species lost to extinction also represents the loss of possible vitamin and protein-rich foods and stable crops. So, more attention is needed to their conservation and sustainable utilization. Conservation of medicinal plants provides sustainable livelihoods as well as the vital protection of biodiversity.

Unchecked commercialization and habitat loss of wild medicinal plants are threatening the beauty, diversity, and natural heritage, as well as the future of vital resources of our planet. This loss of diversity may also take with its important future cures for diseases as potential resources to combat poverty, hunger, and social and economic insecurity. Besides their high medicinal value, these plants have not been cultivated for commercial purposes due to low seed viability, germination rate, and high rate of mortality of seedlings in the early stages. In many threatened plants, *in vitro* propagation is successfully utilized for the conservation of the genetic stocks. *Pseudarthria viscida* is one of such medicinally important plants that need conservation [3].

Pseudarthria viscida (L.) Wight & Arn., which is commonly known as Salaparni in Sanskrit, Moovila in Malayalam, is a perennial viscid pubescent, semi-erect, diffuse undershrub belonging to the family Fabaceae and sub-family Faboideae. The plant is distributed throughout India and is especially found in river basins and in hills up to above 900m. This vulnerable plant has several medicinal uses in the indigenous system of medicine and is an essential component of many famous Ayurvedic formulations like Dashamoolarishta, Mahanarayana taila, Agastya haritaki rasayana, Brahma rasayana, Dhanuanthara ghrita, Anuthaila, Sudarshana churna and Dhantara taila [4]. The most important part is roots, which are used as

digestive, astringent, anthelmintic, anti-inflammatory, thermogenic, cardiotonic, aphrodisiac, febrifuge, nervine, and rejuvenating tonic [4, 5]. It is an important remedy for blood disorders and heart diseases, as it effectively stops bleeding and alleviates edema. Major chemical compounds reported to be present in the roots are 1,5 dicaffeoyl quinic acid, oleic acid, tetradecanoic acid, rutin, quercetin, gallic acid, ferulic acid, and caffeic acid. The roots and leaves contain proteins, tannins, and flavonoids and also showed significant inhibitory activity against some fungal pathogens causing major diseases in crop plants and stored food grains [6]. A polyphenolic compound was reported from the roots of *P. viscida,* which was suggested as the reason for the antioxidant activity of this plant. The plant is used in tridoshas, cough, asthma, fever, dysentery, cardiac ailments, and rheumatoid arthritis and aids in the fast healing of fractured bone. The plant possesses antifungal, antioxidant, antitumor, anti-hypertensive, antidiarrhoeal activity, neuroprotective and anti-inflammatory activity [6, 7]. Due to its high medicinal value, the annual consumption of the root by the Ayurvedic medicine industry in Kerala is 140 tons. Home gardens of *P. viscida* were maintained by the Kani tribe of Kanyakumari Wildlife Sanctuary, Southern Western Ghats [8]. The main aim of this work is to identify effective micropropagation strategies for *P. viscida.*

IN VITRO REGENERATION AND MASS PROPAGATION OF *PSEUDARTHRIA VISCIDA*

The commercial exploitation of medicinal plants has resulted in the reduction of the population of many species in their natural habitat. Therefore, the cultivation of these plants is urgently needed to ensure their availability to the industry and the people associated with the traditional system of medicine. Consequently, it is inevitable to propagate these plants in suitable agroclimatic conditions. *In vitro* propagation is the best method for the conservation of threatening medicinally and pharmaceutically important plants. In the present study, various explants like leaf, internodal and nodal segments isolated from a young, healthy plant were selected for *in vitro* propagation of *P. viscida.* One single medium cannot be suggested for all types of plant tissue and organs. Therefore, the establishment of a new system must fulfil all the specific requirements for the proper growth of a particular tissue because nutritional requirements are essential for optimal *in vitro* growth [9]. Important media used mainly for tissue culture studies were MS medium [9], Gamborg's (B_5) medium [10] and White's mediums [11] *etc*. MS medium was originally formulated to induce organogenesis and regeneration of plants in cultured tissues, whereas, at present, it is widely used for types of culture systems. B_5 medium was originally designed for cell suspension and callus cultures. At present, with certain modifications, this medium is used for protoplast culture and other cell cultures. White's medium was one of the earliest plant tissue culture

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T. Pullaiah

Prof. T. Pullaiah obtained his M. Sc. and Ph. D. in botany from Andhra University. He was a post-doctoral fellow at Moscow State University, Russia, during 1976-1978. He joined Sri Krishnadevaraya University as a lecturer (1979) and became professor in 1993. He held several positions in the University as Dean, Faculty of Life Sciences; Head of the Department of Botany; Chairman of the Board of Studies in Botany; etc. In 2011, he was selected by UGC as UGC-BSR faculty fellow in the Department of Botany, Sri Krishnadevaraya University. He has published 120 books, 340 research papers and 35 popular articles. His books have been published by reputed international publishers like Elsevier, Springer, CRC Press, Taylor & Francis, Bentham Science, Apple Academic Press, Scientific Publishers, Astral International, CBS Publishers etc. He was the principal investigator of 20 major research projects. Under his guidance, 54 students obtained their Ph. D. degrees and 34 students their M. Phil. degrees. He is the recipient of the Best Teacher Award from the Government of Andhra Pradesh, Prof. P. Maheshwari Gold Medal, Prof. P.C. Trivedi medal for Editorial excellence and Dr. G. Panigrahi Memorial Award of Indian Botanical Society etc. He was the president of Indian Association for Angiosperm Taxonomy (2013) and president of the Indian Botanical Society (2014). He was a member of Species Survival Commission of International Union for Conservation of Nature and Natural Resources (IUCN).