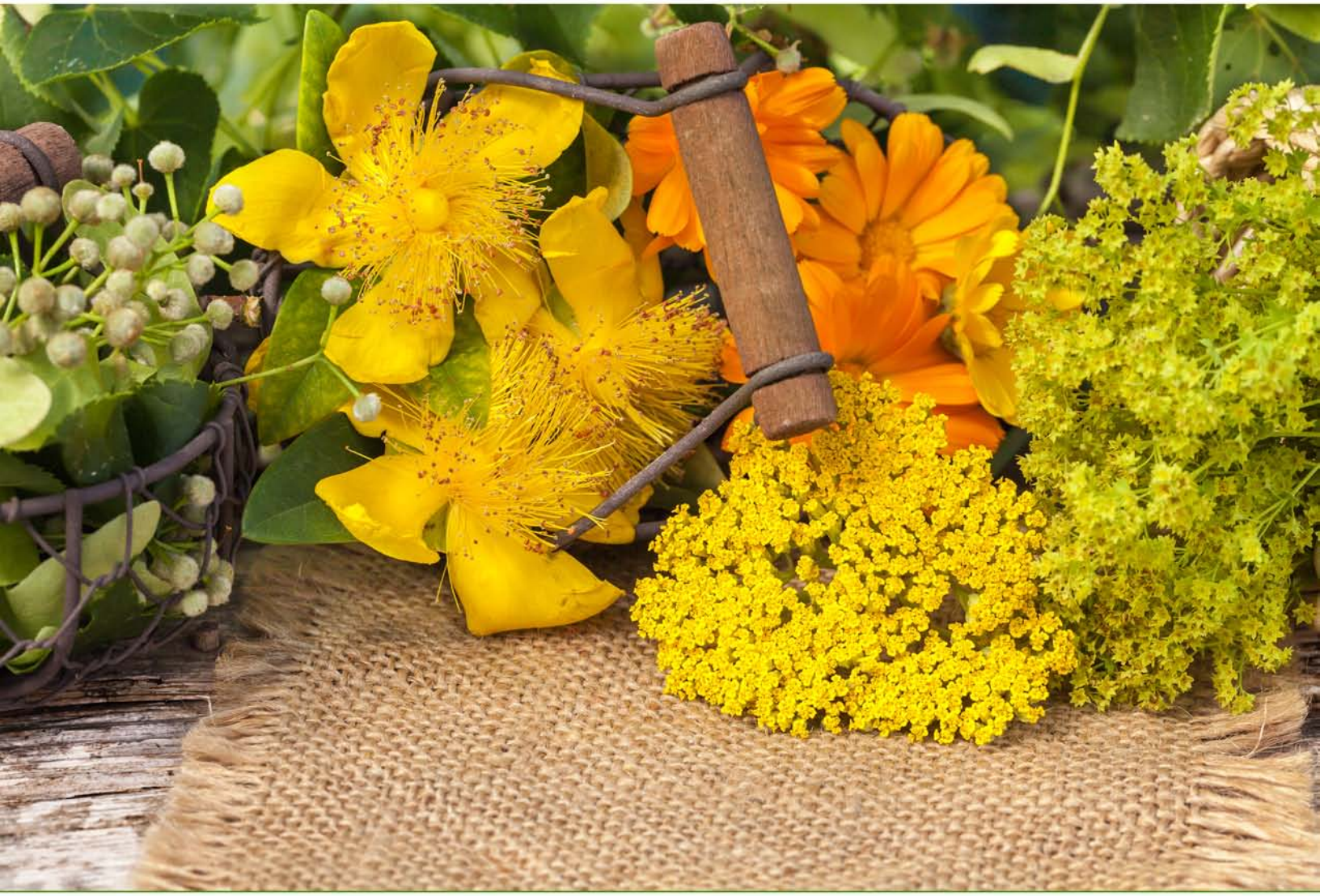
MICROPROPAGATION OF MEDICINAL PLANTS



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PREFACE

The cultivation of medicinal plants, especially high-value medicinal plants, is creating a new dimension in the field of agriculture. However, the cultivation of medicinal plants is not easy. It is a challenging task because of very little knowledge of seed and pollination biology, nutrient and microhabitat requirements, pest management and growing seasons. Not much effort has been made to search for elite specimens and their propagation.

In recent years, plants in their natural habitat have become rare due to large-scale destruction for medicinal uses, long intervals for vegetative, flowering and fruiting stages, short viability of seeds and low seed germination. It is, therefore, important to conserve these medicinal plants because of their commercial importance. Conventional propagation methods cannot replace the depleting population because the seeds show a low percentage of germination, and vegetative propagation methods are sometimes unsuccessful. The development of standardized micropropagation techniques for the improvement of medicinal plant species is an important issue for preserving biodiversity. Further propagation through seeds may not fulfil the commercial demand of herbals and therapeutically important medicinal species. *In vitro* culture can be a valuable technique for clonal mass propagation and conservation of these medicinal plants within a short period of time.

Micropropagation, however, is highly labour oriented and, thereby, commercial companies are outsourcing plant multiplication activities to low-labour cost areas. Hence, in technologically advanced countries, the great potential of micropropagation for large-scale plant multiplication can be tapped by cutting down the cost of production per plant, pursued by applying the low-cost tissue culture, adopting practices, and optimizing the use of equipment and resources to reduce the unit cost of micropropagule and plant production without compromising the quality. Furthermore, the development and rapid multiplication of new medicinal plant cultivars are required to meet the demand of the industry all year round. The existing and refined protocols for *in vitro* culture, as well as their direct applications in improving and developing new cultivars, regularly supply plant material year round. Moreover, *in vitro* long-term storage of valuable germplasm would immensely provide benefits to both the industry and academic institutes. The outcome of recent studies carried out in various research laboratories and institutions shows optimized micropropagation protocols for many medicinally-important species and well-developed *in vitro* techniques, such as thermotherapy and cryotherapy for virus-free production, exploitation of somaclonal variation, long-term shoot culture conservation, and plant rejuvenation.

The present book gives the protocols for micropropagation of more than 40 species of medicinal plants. This book smartly combines scientific principles with the state-of-the-art in tissue culture techniques presented by experienced 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 sourcebook 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

Biotization of Medicinal Plant Cultures by Endophytes: A Promising Approach to Enrich Therapeutics

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Abstract: Overexploitation, climate change, and pressure from invasive species are threatening the diversity of medicinal plants; a few of them are extinct or in the endangered category. The mass multiplication of some medicinal plants outside their natural habitat affected the biochemical diversity of the plants, thereby decreasing their medicinal value. Hence, micropropagation of high-yielding, elite genotypes was preferred over time to conserve the species and meet the pharmaceutical needs. Although micropropagation was promising, the diversity and quantity of bioactive compounds of the *in vitro* plants were not comparable to those of their counterparts in nature. The *in vitro* plants, challenged with a plethora of biotic and abiotic stresses, were poorly acclimatized, with abject survival. During the last few decades, the role of endophytes with their mechanisms in enhancing growth, development, and stress tolerance has been proven among field-grown plants. In consequence, the role of endophytes in micropropagation is gaining prominence to address the vulnerability, acclimatization, and enhanced bioactive compounds of tissue culture plants. This approach of the use of competent endophytes is known as biotization. This chapter brings together the current status, possibilities, and limitations of the most promising biotization of medicinal plants. Biotization of endophytes in micropropagation is a potential tool for the production of medicinal plants with enriched bioactive compounds with improved therapeutic effects.

Keywords: Acclimatization, Bioreactors, Biotization, Cell and tissue culture, Endophytes, Medicinal plants, Secondary metabolites, Stress tolerance.

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T. Pullaiah (Ed.)

INTRODUCTION

Need for Micropropagation of Medicinal Plants

Medicinal plants have been an important source of human therapy since time immemorial. All over the world, 70-80% of people use herbal medicines [1]. Although the exact number of medicinal plants in the world is not known, about 50,000 species are used in traditional and allopathic medicines, of which ⅔ are harvested from their natural habitat [2]. The plants are harvested extensively for various purposes: (1) For veterinary purposes and the well-being of livestock (2) In folk medicine, for snake bite, and anti-ophidic activity (3) for traditional medicine, and (4) Allopathy [3, 4] (Table **1**). Additionally, some of the trees, like cancer trees and guggle trees, are used for wood, package, and gum purposes, without the awareness of the medicinal properties of such trees, and the fact that they are in critically endangered condition (Table **1**). An added threat to the medicinal plants is their use to treat antibiotic resistance. Antibiotic resistance is reported all over the world, caused by the misuse or repeated use of antibiotics [5]. In attempts to treat patients with multiple drug resistance (MDR), plant extracts are in practice, which is a further provocation to the medical plants.

Table 1. Micropropagation of popular medicinal plants with genetic fidelity tested through molecular methods.

	Plant Species	Metabolite	Medicinal Value	Therapy	Micropropagation References
1.	*Aegle marmelos* (L.) Corr. Bael	Furocoumarin Marmelosin, aegelionol	Laxative and diuretic	Ayurvedha Unani	Pati *et al.* [38]
2.	*Acorus calamus* L.	β-asarone	sedative, anti-diarrheic, carminative, tonic, stimulant	Traditional	Babar *et al.* [39]
3.	*Artemisia nilagarica* (C.B.Clarke) Pamp. var. *nilagarica* *A. absinthium* L. Indian wormwood	Artemisinin	Anti-malarial, antihelminthic, insecticidal, antiseptic, and antibacterial	Traditional medicine	Shinde *et al.* [40] Kour *et al.* [41]
4.	*Andrographis paniculata* Nees King of bitters	Andrographolide	Anti-bronchitis and anti-cancer	Traditional medicine	Dandin and Murthy [42]

(Table 1) cont.....

	Plant Species	Metabolite	Medicinal Value	Therapy	Micropropagation References
5.	*Spilanthes oleracea* L. Toothache plant	Scopoletin	Toothache, stammering, and stomatitis	Traditional medicine	Dandin *et al.* [43]
6.	*Celastrus paniculatus* Willd. Jyotishmati	Celapagine, celapanigine, and celapanine	Treating skin diseases	Abortifacient, antidote for opium	Senapati *et al.* [44]
7.	*Catharanthus roseus* (L.) G.Don	Catharanthine vincristine and vinblastine.	Anticancer	Allopathy	Kumar *et al.* [45]
8.	*Campotheca acuminata* Decne Cancer tree/happy tree	Camptothecin (CPT) inhibitor of Topo-I	Anticancer, antiviral (HIV)	Allopathy	Sankar [46]
9.	*Nothapodytes nimmoniana* (Graham) Mabb.	Camptothecin (CPT)	Anti-cancer	Allopathy	Prakash et al., 2016 [47]
10.	*Commiphora wighiti* (Arn.) Bhandari Guggul tree or Myrrh tree	Guggulsterone-E and Z	Obesity and lipid metabolism	Ancient Ayurvedic medicine	Parmar and Kant [48]
11.	*Curcuma zedoaria* (Christm.) Roscoe	Curcumin	Anti- cancer	Indian Ayurvedic medicine	Jena *et al.* [49]
12.	*Curcuma angustifolia* Roxb.	Curcumin	Anti- cancer	Indian Ayurvedic medicine	Jena *et al.* [50]
13.	*Curcuma longa* L	Curcumin	Anti- cancer	Indian Ayurvedic medicine	Pittampalli *et al.* [51]
14.	*Narcissus tazzeta* L. var. *chinensis* Roem	Galantamine	Anti-alzheimer	Allopathy	Chen *et al.* [52]
15.	*Pilocarpus microphyllus* Stapf	Pilocarpine	Anti-glaucoma	Allopathy	De Abreu *et al.* [53]
16.	*Piper aduncum* L.	Piperine	Antioxidant	Allopathy	De Sousa *et al.* [54]
17.	*Piper longum* L.	Piperine	Antidiabetic,	Indian Ayurveda	Chatterjee *et al.* [55]

CHAPTER 2

In vitro Propagation Protocol of *Tylophora indica* (Burm.f.)Merrill

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Abstract: *Tylophora indica* (Burm.f.) Merrill is one of the most commonly used medicinal plants with bioactive alkaloid-rich secondary metabolites. This plant is used to treat asthma, dysentery, whooping cough, rheumatic pains, jaundice, and cancer. Rapid exploitation of this plant in natural habitats and poor regeneration methods, which are not in pace with those of destruction, make tissue culture methods a viable option to be used as a method of conservation. In the present chapter, tissue culture protocols have been reported till now as the best viable means in the rapid multiplication of *T. indica*. Sterilization protocols, callus induction and somatic embryogenesis methods, and direct and indirect organogenesis used by different researchers in mass propagation and acclimatization are given in detail. The present chapter gives an insight into the hormones needed and the response of the explants, which will be helpful for those who want to propagate this medicinal plant under *in vitro* conditions.

Keywords: *In vitro* propagation, Micropropagation, *Tylophora indica*.

INTRODUCTION

Tylophora indica (Burm.f.) Merrill [Syn.: *Tylophora asthmatica* (L.f.) Wight &Arn.], commonly known as Indian ipecac, is a medicinal plant belonging to the family Apocynaceae. It is distributed in Malaysia, Sri Lanka, and India to South-East Asia. It is a profusely branched, perennial climbing herb. It grows well in sandy soils, and moist and humid conditions, with flowering and fruiting from August to December. The leaves and roots of this plant have medicinal properties and are used to treat asthma, diarrhoea, bronchitis, dysentery, whooping cough, *etc* [1, 2]. The bioactivity of *Tylophora indica* was due to the presence of different alkaloids such as tylophorinicine, tylophorine, O-methyl tylophorinidine, septicine,isotylocrebrine, *etc* [3 - 9]. The presence of tannins, resins, phenols,

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saponins, terpenoids, sterols, wax, and flavonoids was also reported by many researchers [10 - 15].

The significance of this plant was due to its high medicinal importance [2, 16 - 20]. This plant has been used in Ayurveda for treating asthma, dermatitis, and rheumatism [21, 22]. The leaves of this plant are used to treat asthma [23, 24] and myocardial damage [25]. The plant parts possess anti-inflammatory activity [26, 27], anticancer activity [5, 9, 28 - 33], antiallergic activity [34], hepatoprotective activity [35, 36], antimicrobial activity [4, 15, 37, 38], diuretic activity [10], and antidiarrhoeal [39], antihyperglycemic and anti-hyperlipidemic activities [40]. It reduced alcohol-induced anxiety in Wistar albino rats [41]. Roots also contain tylophorinidine alkaloid, so it can also be used as an anticancer and anti-inflammatory agent [5, 6].

Even though the seed germination was high, fruit setting is a rare phenomenon, it can be propagated through vegetative propagation, but improper rooting [42] was a major drawback. Large-scale collection of this plant and insufficient replenishment methods created the need to use tissue culture methods for its rapid propagation [43 - 45]. Initial studies of tissue culture in *T. indica* were restricted to alkaloid synthesis using callus [46 - 50]. Rao and Narayanaswamy [47] used stem explants and carried out their investigations to study the process of embryogeny and compared it with normal patterns. They have also studied the optimal nutrients needed and changes in tissue differentiation. Benjamin *et al.* [50] cultured leaf, stem, and roots for callus induction and synthesis of alkaloids. They have reported that the origin of the tissue, hormonal stimuli, and organogenesis did not influence alkaloid synthesis. Mhatre *et al.* [51] attempted for protoplast cultures of *T. indica.* They have reported that when they used a 5-year-old callus for protoplast culture, it divided without shoot bud differentiation, whereas the fresh protoplast-derived callus formed shoot buds and embryoids. Sharma and Chandel [52], for the first time, reported shoot induction from axillary shoot sprouting.

Anand *et al.* [53] used *in vitro* grown leaves, callus, and suspension cultures for extracting plant extract. Extraction was done using a cold extraction method with acetic acid in methanol followed by acid extraction with ethyl acetate: HCl (hydrogen chloride). Later, tylophorine was separated using High-performance thin-layer chromatography (HPLC). After a quantitative study, they reported 80 micrograms per milliliter (µg/ml) of tylophorine in leaves, 28.30µg/ml in suspension cultures, and 24.46µg/ml of tylophorine in callus cultures. Rathod *et al.* [54] carried out investigations regarding the biochemical changes that occur during *in vitro* regeneration of callus and shoot formation from this callus. They have observed increased peroxidase, polyphenol oxidase, and Indoleacetic acid

(IAA) oxidase activities with differentiating green callus. They have also observed a decrease in reducing sugars and protein content and an increase in metabolic rate during *in vitro* organogenesis. Soni *et al.* [55] incorporated tyrosine in the growth medium, which is a precursor for the production of tylophorine. They reported an enhanced tylophorine production (27.7micrograms per gram (μg/g)) with 2mg/L tyrosine incorporation. They also noticed 5.87-fold higher levels of tylophorine from *in vitro*-raised plants when compared with that of *in vivo* plants. Kaur *et al.* [56] also used HPLC for tylophorine purification using toluene: chloroform:ethanol: ammonia (4:3.5:1.5:drop). They reported 80μg/ml tylophorine in leaf callus regenerated plants, while with direct organogenesis, plants contained 71 μg/ml of tylophorine. Gantait *et al.* [57] reported single bead alginate encapsulation of *T. indica in vitro* developed nodal segments, obtained after culturing them on the medium reported by Faisal *et al.* [44] for 40 days. Encapsulation was achieved using 75 millimoles per liter (mmol/l) calcium chloride ($CaCl_2$) + 3 percent (%) weight in volume (w/v) sodium alginate. They kept these artificial seeds at 15±1degree Celsius (^oC) for 15 days and found a 90% of conversion frequency. They have also observed that this conversion frequency has decreased to 70% after 30 days of storage.

MICROPROPAGATION

Micropropagation studies carried out on *Tylophora indica* are tabulated in Table **1**.

Table 1. Overall *in vitro* propagation work done till now in *Tylophora indica*.

S.No	Explant	Medium, Hormones, and Supplements	Response Observed	References
1	Stem	Modified MS medium+2,4-D (2mg/L)+CM (10%)+ CH (200mg/L)+inositol (100mg/L) adenine (5mg/L) IAA (0.5mg/L)	Callus, shoots, embryos Roots	[46]
2	Internodes	White's medium+ 2,4-D (1mg/L) 2,4-D (0.1mg/L) or devoid of 2,4-D + CH (500mg/L)	Callus and proembryos Embryos converted into plants	[47]
3	Seedling stem, root	MS medium +2,4-D (2mg/L)+ CM (10%) + CH (200ppm)+ adenine (5ppm)	Callus	[48]
4	Stem	MS medium +2,4-D (2mg/L)+ CM (10%) + CH (200ppm)+ adenine (5ppm)	Callus, gamma ray irradiation effect	[49]

CHAPTER 3

A Review of Tissue Culture Studies on *Withania somnifera* (L.) Dunal - An Important Medicinal Plant

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Abstract: *Withania somnifera*, commonly known as 'Indian ginseng', is a highly important and valuable medicinal plant in traditional family medicine, containing a variety of medicinal bioactive molecules for over 3,000 years. Various medicinal properties of plants are attributed to steroidal lactones (withanolides) present in plants. Its commercial cultivation is hampered by low seed viability and germination rates. Tissue culture techniques can play an important role in the preservation, clonal propagation, and qualitative improvement of this medicinal plant. *In vitro* shoot differentiation and micropropagation of *W. somnifera* from various small excised explants such as hypocotyl and cotyledon leaves, shoot tips, nodes and internodes. Optimal normal growth, reproduction, and development of *W. somnifera* through the *in vitro* processes of morphogenesis of many tissues may differ in several different plants based on key plant nutrient requirements. The current review provides a comprehensive study on the development of *W. somnifera* tissue culture research activity. It also discusses the medicinal properties of this plant.

Keywords: *Withania somnifera*, Micropropagation, Tissue culture, *In vitro* propagation.

INTRODUCTION

Higher plants are the ultimate renewable, solar-powered biochemical factories producing both primary and secondary metabolites from air, water and minerals.

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This has led humans to manipulate and modify plants to meet their growing needs for food, industrial raw materials, pharmaceuticals, pesticides, flavours and fragrances. The continued use of herbal medicines by a large portion of the population in developing countries is largely due to the high cost of western medicines and medical care. Therefore, the recognition and development of the medicinal and economic benefits of these plants are increasing in both developing and developed countries. The herbal industry is in its prime, but growing medicinal plants is not easy. Micropropagation is one of the rapid and most reliable propagation techniques for most medicinal plant species. Although reports of *in vitro* culture of this valuable medicinal plant are available, efficient protocols for improved cultivar propagation are needed, as the *in vitro* responses of plant species different from cultivar to cultivar. In particular, growth hormone requirements for each cultivar must be empirically determined as they are responsible for the majority of the physiological activity of the crop. However, it is of commercial importance that drives research start-ups. Based on their relative potent importance, *Withania* (commonly known as Indian ginseng, Ashwagandha in Hindi and Sanskrit, and winter cherry in English) contains a number of pharmacologically important active withanolides, withaferin -A (WS-3). It has been used as a sedative, astringent, stimulant, aphrodisiac, diuretic and tonic, as elucidated in Ayurveda. There are now several forms of Withania pure herb and herbal extracts that are marketed worldwide as herbal GRAS (Generally Recognized as safe) products that promote health. *W. somnifera* is also known as a vital medicine of Siddha and Unani medicine.

Of the 23 known *Withania* species, only two (*Withania somnifera* (L.) Dunal and *Withania coagulans* Dunal) are commercially important [1]. It is a medium-sized shrub, branched perennial, about 30 cm to 1.5 m tall. It is found in the drier regions of India and widely distributed in tropical and subtropical regions of the world, including India [2]. In India, the species is native to the North Western regions and extends up to 1500 meters altitude in the foothills of Punjab, Himachal Pradesh, Jammu and Kashmir [2]. It is a medicinal plant widely used in India and other parts of the world. It is a vital ingredient in over 100 traditional pharmaceutical formulations [3]. *W. somnifera* comprises a number of pharmacologically energetic secondary metabolites known as pyrazole alkaloids, withasomnin, steroidal lactones, withaferin-A, withanolide-D, and withanolides, which are valuable pharmaceuticals [4, 5]. The plant has numerous medicinal benefits such as anti-inflammatory, anticancer, antistress, antiaging, adaptogenic, and immunomodulatory properties and exhibits free radical scavenging activity. It is also used to treat tuberculosis, rheumatism, inflammatory diseases, and heart disease. It is also useful as an abortifacient, amoebicidal, analgesic, bactericidal, contraceptive, and antispasmodic. Roots are used as anti-aging agents and for the prevention and control of Alzheimer's disease [6 - 9]. Dharajiya *et al.* [10]

analyzed the antibacterial activity of various extracts of *W. somnifera* strains against the Gram-negative bacteria *Escherichia coli, Serratia marcescens*, *Pseudomonas aeruginosa*, and the Gram-positive *Bacillus cereus*. This has also been considered an excellent nerve tonic [11]. Ashwagandanolide, a novel dimeric withanolide derived from ashwagandha tuber root, has shown utility against human gastric, breast, central nervous system, colon, and lung cancer cell lines [12]. These properties make ashwagandha one of the most popularly used medicinal important plants [13]. An improved cultivar 'Posita' was released by the Central Institute of Medicinal and Aromatic Plants, Lucknow, India, to meet the needs of the pharmaceutical industry and local practitioners [14]. These cultivar features have better root yield and excellent biochemical constituents.

CHEMICAL COMPOUNDS IN *WITHANIA SOMNIFERA*

The main biochemical constituents of ashwagandha root are steroidal alkaloids and steroidal lactones, a class of constituents called withanolides [15]. To date, 12 alkaloids, 35 withanolides and several sitoindosides have been isolated and characterised from this important plant. Sitoindosides are withanolides containing a glucose molecule at carbon 27. Much of the pharmacological activity of ashwagandha is attributed to the two major withanolides, withaniferin-A and withanolide D. Withanolides function as important hormone precursors that can be converted into human physiological hormones as needed. Ashwagandha is thought to be amphoteric and helps regulate important physiological processes, showed that the actual hormones become attached and unable to exert their effects. When hormone levels are low, herbal hormones have a little effect. They are the main bioactive components of ashwagandha root.

Withanolides are the nine and the most abundant groups of the broad parental group of Withasteroids. Withanolides can be classified into two classes: (1) with normal 17 β-oriented chain (132 compounds known), (2) with the unusual 17 α-oriented chain (36 compounds known). Rockley *et al*. [16] showed that 24-methylene-cholesterol, rather than its double bond isomer, is the true precursor of withanolides.

The first member of this group, withaferin-A (WS-3), was isolated from *W. somnifera* in 1965 [17]. Quantitative estimates of withaferin-A in leaves and roots of *W. somnifera* are 1.13% and 0.044% [18]. The roots of *W. somnifera* contain four steroidal lactones called withanolides, namely withaferrin-A 5,20a(R)-dihydroxy-6a,7a-epoxy-1-oxo-(5a)-witha-2,24-dienolide and two small with-anolides, probably 5a,17a-dihydroxy-1-oxo-6a,7a-epoxy-22R-witha-2,24-dienolide (so-called withanone) [19].

CHAPTER 4

Micropropagation of *Aloe vera*

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Abstract: *Aloe vera* is a medicinal plant with several properties and is used in pharmaceutical, medicinal, biomaterials, food, and cosmetic industries. This plant is associated with hot climates and dry habitats, with plantlet production based on lateral shoot propagation, an expansive and slow method, insufficient to meet the increasing demand of the industry. Therefore, the development of a suitable *Aloe vera* micropropagation protocol is crucial, keeping the genetic integrity and providing large-scale plantlets production. Nevertheless, parameters like source of plant tissue, surface-sterilization process, culture medium conditions and plant growth regulator concentration can affect the morphogenic response process. Since all parameters are defined to obtain the best performance, the micropropagation protocol is suitable to be used commercially, providing mass production of *Aloe vera* plantlets with high quality.

Keywords: *Aloe vera*, micropropagation, medicinal plant.

INTRODUCTION

Comprising about 548 species and belonging to the family Xanthorrhoeaceae, the *Aloe* genus is associated with hot climates and dry habitats, useful for ecosystem restoration, and a few *Aloe* species are used as a source of herbal medicines or food [1]. *Aloe vera* is an important commercial crop with several species and varieties in international markets, widely used for medicinal purposes in different countries like Brazil, India, China and Japan [2]. These medicinal properties can be attributed to the leaf gel present in the outer layers [3], which is widely used by cosmetic, pharmaceutical and food industries [4, 5]. This species has high water content (99%), and contains over 75 different active compounds, like minerals, vitamins, polysaccharides, enzymes, phenolic compounds and organic acids [6].

The conventional propagation of *Aloe vera* seedlings by axillary shoot is very slow [7] and inefficient to provide a high production to industrial demand [8], mainly to fast growing demand of cosmetic and pharmaceutical industries [9].

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The low propagation of this species is due to limited seed production [10], the presence of male sterility [11] and a limited number of lateral shoots produced from a single plant [9]. To improve agronomic and medicinal traits, conventional plant breeding methods are widely applied. Nevertheless, the plant tissue culture technique presents high potential for the production of high-quality plant-based medicines [12].

Therefore, the best viable technique to produce healthy plants in large numbers and in a short time is through plant tissue culture. This technique is used to culture tissues and organs of plants to obtain disease-free clones, and also for plant improvement and mass plant multiplication. Although some tissue culture protocols for *Aloe* spp. have been developed [13 - 15], an efficient regeneration protocol is necessary for mass production of this commercially important species [2].

ALOE VERA APPLICATIONS

The main *Aloe* species used in the cosmetic industry are *A. vera* and *A. ferox*, generally used in health and skincare products [16, 17]. The gel obtained from the leaves (leaf gel) has several properties and active ingredients [18] and is used in the production of shampoos and skincare creams.

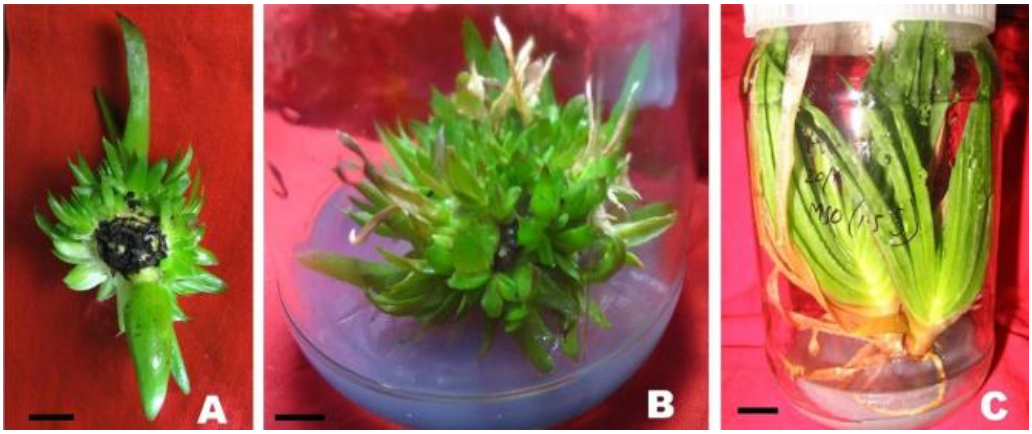

Fig. (1). Micropropagation of Aloe vera. A, B - Shoots induced; C - Plantlets with root system. bar = 1 cm. Source: Haque and Ghosh, Botanical Studies 2013, 54:46.

In the pharmaceutical/medicinal industry, very few *Aloes* species are used, like *A. vera, A. ferox* and *A. perryi* [19]. Some chemical components from *Aloe* leaf gel have important activities useful for medicinal applications, like healing skin activity, protection of liver and kidney, treatment of infection of eyes [20 - 22] and anti-cancer agent [23]. In addition, according to Radha and Laxmipriya [6], *A. vera* has an effect on burn wound healing, stimulating the proliferation of several

cell types and increasing the collagen synthesis and wound contraction; on immunomodulatory activity, inhibiting the inflammatory process followed by burn injury; on intestinal absorption, being used for drug absorption enhancement for drugs with low bioavailability; on antidiabetic treatment, acting as a safe antihyperglycemic and antihypercholesterolemic agent for type 2 diabetic patients; on antioxidant effect, due to substantial amounts of antioxidants content, as vitamin E, carotenoids, vitamin C, flavonoids, and tannins; on hepatoprotective activity, inducing the downregulation of fatty acid synthesis and upregulating the fatty acid oxidation in the liver, improving the hyperlipidemia; on anticancer activity, due to the aloin content, a natural compound with high potential therapeutic options in cancer treatment; on antimicrobial activity, producing anthraquinones as an active compound, which is structural analogue of tetracycline; on antiviral activity, producing anthraquinone derivatives, such as aloe-emodin, emodin, and chrysophanol, preventing virus adsorption, attachment, or entry to the host cell; and on antiulcer activity, whereby *A. vera* gel expresses antibacterial properties against both susceptible and resistant *Helicobacter pylori* strains.

In the biomaterials industry, recent advances show a high potential of *A. vera* for tissue engineering applications due to several advantages as biocompatibility, oxygen permeability, antioxidant action, effect on cell proliferation and regeneration, biodegradability, and low toxicity effects [24].

ALOE VERA TISSUE CULTURE

Different protocols have been developed for micropropagation of this species, and the best protocol to be used depends on the researcher's objective. Some modifications can be performed to reach different results as better shoot multiplication, rooting process, less contamination or a high survival rate.

In some studies of micropropagation of this species, *Aloe vera* leaf gel (AvG) was used in the shoot multiplication/rooting medium. This compound contains over 75 active ingredients, such as enzymes, minerals, different inorganic salts, amino acids, and salicylic acids, acting as a nutritional supplement [18]. To improve the seedlings production ratio by micropropagation, the *in vitro* proliferation can be significantly increased, subculturing the microshoots multiplied [7]. Furthermore, it is possible to improve the *Aloe vera* micropropagation, increasing plant biomass, radical scavenging capacity, and chlorophyll, phenol and gel content, using the symbiotic fungus *Piriformospora indica* [25].

It is important to note that the micropropagation system for commercial production needs to present genetic fidelity. To this purpose, some DNA-based markers, such as SSR (simple sequence repeats) and RAPD (Random Amplified

CHAPTER 5

Micropropagation protocols for *Phyllanthus amarus* Schum. & Thonn.

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Abstract: *Phyllanthus amarus* Schum. & Thonn. is a medicinal plant belonging to the family Phyllanthaceae. It is used in Siddha, Homeopathy, Unani and Ayurvedic medicines. This plant is used for treating skin diseases, diabetes, fever, diarrhea, gastric problems and migraine. It also acts as an anticonvulsant, anti-inflammatory, analgesic, antiamnesic, antimicrobial, nephroprotective, antiviral, hepatoprotective, anticancer, and anti-atherosclerotic agent. It grows as a weed, but because of its high medicinal value, this plant was propagated *in vitro,* so as to meet the market demands and for rapid propagation. In the present review, detailed work done till now is discussed, with different methods of sterilization, and hormones used for shoot, callus and root induction.

Keywords: Micropropagation, *Phyllanthus amarus*, shoot induction, root induction.

INTRODUCTION

Phyllanthus amarus Schum. & Thonn. is a medicinal plant belonging to the family Phyllanthaceae and grows as a weed. This plant is a herb, growing to a height of 60-75 cm. It is a native of tropical America, now is a pantropical weed. It grows on sandy loam soils. It is distributed throughout India and used in Siddha, Homeopathy, Unani and Ayurvedic systems of medicines [1, 2]. Stem is angular, leaves are elliptic-oblong, flowers are unisexual and axillary, yellow or green in colour. Fruit is a globose capsule underneath branches. Extracts of this plant contain flavonoids, polyphenols, tannins, alkaloids, lignans (phyllanthin and hydrophyllanthin), triterpenes, and sterols [3 - 10]. Its medicinal properties are utilized worldwide. It is used to treat skin diseases [11, 12], antidiabetes [13, 14], antimicrobial [15 - 17], anti-inflammatory [18], anticonvulsant [19], hepatoprotective [20 - 22], analgesic and antipyretic [23 - 26], antiviral [27, 28],

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nephroprotective [29], antiamnesic [30, 31], antidiarrhoeal, gastroprotective, antiulcer [32 - 34], antimigraine [35], antiatherosclerotic [36], anticancer [37] and antileptospiral [38, 39].

MICROPROPAGATION PROTOCOLS

Bhattacharyya and Bhattacharya [40] cultured shoot tips of *Phyllanthus amarus* on Murashige and Skoog (MS) medium containing 6- Benzylaminopurine (BAP) and Kinetin (Kn) for direct shoot regeneration. Later, these two researchers attempted to develop hairy roots of *P. amarus* using *Agrobacterium rhizogenes* [41]. They studied the ability of this plant in the inactivation of hepatitis B virus surface antigen. Ravindhran *et al*. [42] reported shoot tip cultures and plantlet development procedures. Singh *et al*. [43] reported synthetic seed formation using the shoot tip of *P. amarus*. Shekhawat and Dixit [44] attempted for shoot culture using nodal segments. Thakur and Kharya [45] attempted to enhance the production of bioactive compounds of *P. amarus*, using the immobilization technique. Phyllanthin and hypophyllanthin have a hepatoprotective nature and their supply in *in vivo* is very low, so to increase the supply of the bioactive components, these scientists have used calcium alginate beads. They have also noticed that bioactive compounds production was enhanced by using MS medium and phytohormones like 5 percent (%) Kn, 88% 1- Naphthalene acetic acid (NAA), 238% Chitosan and 252% Cinnamic acid as precursors (individually) in immobilization process when compared with control (0.12%). High performance thin layer chromatography (HPTLC) analysis was used to check the increase in these compounds. Cinnamic acid addition in MS medium has increased production of phyllanthin and hypophyllanthin 0.422 weight by weight (w/w) and was found to be the best among tested treatments. Later, they attempted for immobilized cell cultures using MS medium. They tried to increase the production of bioactive contents like phyllanthin and hypophyllanthin using phenylalanine (41.3%), chitosan (238%), copper sulphate (367%) and silver nitrate (654%). Cell viability was assessed using UV fluorescence microscope and found 66% of *P. amarus* cells viable in immobilized cultures. They have reported silver nitrate to be the best abiotic elicitor, among the selected 4 in their experimental study. Shetti *et al*. [14] experimentally proved the antidiabetic effect of the ethanolic leaf extract of *P. amarus,* using alloxan induced diabetic mice.

Explant Sterilization

Bhattacharyya and Bhattacharya [40] washed shoot tips obtained from one-month-old plants thoroughly with tap water and with a few drops of Sursol detergent. Later, treated with alcohol (70%) for 2 minutes (min), then treated with a mixture of bavistin (0.1% w/v) and streptocycline 0.2% weight in volume (w/v)

for 1 hour (h). After each treatment, explants were rinsed with sterile double-distilled water for 3-4 min. Ghanti *et al*. [46] washed explants first with running tap water and a few drops of liquid detergent. Later, disinfected with 0.1% mercuric chloride ($HgCl_2$) for 1-2 min and rinsed with sterile distilled water. Ravindhran *et al*. [42] sterilized shoot tips under running tap water for half an hour and then treated with 70% alcohol for 2 min and 0.1% (w/v) bavistin and 0.1% $HgCl_2$ for 3 min each. Later, rinsed with sterile double-distilled water for 3-4 times. Marimuthu and Antonisamy [47] collected internodal segments of mature plants and washed under running tap water for 5 min, then with tween-20 for 3 min, then rinsed with sterile distilled water. Later, treated with $HgCl_2$ (0.5% w/v) for 2 min, rinsed thrice with sterile distilled water.

Chitra *et al*. [48] sterilized leaf bits (0.5- 1.0 centimeter square (cm^2)) and internodes (1.0- 1.5 centimeter (cm)) washing with Teepol for 2 min and washed thoroughly under tap water for 3 min. Later, disinfected and treated with 0.1% $HgCl_2$ for 2 min and washed with sterile distilled water 4-5 times. Sen *et al*. [49] sterilized nodal explants under running water for 15-20 min to remove soil particles and then treated with Teepol (1%) volume by volume (v/v) for 5-10 min. Rinsed 3-4 times with sterile double-distilled water and treated with $HgCl_2$ (0.1% w/v) for 2 min. Finally, rinsed with sterile water thoroughly. Nitnaware *et al*. [50] sterilized nodal and leaflet explants (10 mm) with tap water followed by 0.1% $HgCl_2$ treatment for 5 min and washed thoroughly with sterile distilled water. Thakur *et al*. [10] and Thakur and Kharya [45] sterilized leaves with running tap water wash first and 2% tween wash followed by treatment with 70% ethanol volume by volume (v/v) and 0.1% $HgCl_2$. They later used these leaves to collect the bioactive compounds using an immobilization technique. Sen and Batra [51] used nodal segments of field grown juvenile plants and washed under running tap water for 30 min and treated with teepol (1%v/v) for 10 min. Later rinsed with sterile double-distilled water thrice and disinfected with $HgCl_2$ (0.1% w/v) for 2 min. Finally, rinsed with sterile water for 3-5 times.

Xavier *et al*. [52] first washed the selected explants - nodes and shoot tips- in running tap water with Tween 20 for 5 min and washed with distilled water. Later washed the explants with alcohol for 30 seconds (sec), 0.1% $HgCl_2$ for 5 min and washed with sterile distilled water thoroughly. Raja [53] sterilized nodal segments by washing thoroughly under tap water for 15 min and treating with 5% Teepol and 0.1% Bavistin for 5 min. He later surface sterilized with 0.1% $HgCl_2$ for 4 min and washed with sterile double-distilled water thoroughly. Elamvaluthi *et al*. [54] sterilized selected explants with running tap water for 10 min and then treated with double-distilled water containing 3 drops of Tween-20 detergent and sodium hypochlorite for 8.5 min, then treated with distilled water thoroughly and 0.1% $HgCl_2$ for 2-3 min. This was followed by treatment with 70% alcohol and 3-

CHAPTER 6

Micropropagation and *In Vitro* Studies in *Hedychium* J. Koenig (Zingiberaceae)

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Abstract: *Hedychium,* a tropical to subtropical Asian genus with about 100 species, has various medicinal and horticultural uses. There is a high rate of exploitation and disappearance of its species from natural habitats. Additionally, habitat loss and natural calamities should speed up the erosion of this plant species. Micropropagation is considered a multiplication and conservation strategy for medicinal plants. Micropropagation in *Hedychium* is very scanty, and protocols have been developed only for less than 20 species so far. *Hedychium coronarium* and *H. spicatum* are well-studied species *in vitro* among the micropropagated species. It is interesting that micropropagations through protocorm-like bodies were achieved in *H. coronarium*. The selection of explants and their axenic development *in vitro* is the major hurdle in micropropagation. Cotyledonary nodes, shoot tip or shoot tip meristems from axenically germinated seeds, rhizome buds, rhizome meristem, and zygotic embryos were the explants commonly used for the micropropagation of *Hedychium*. Various *in vitro* methods such as somatic embryogenesis, direct organogenesis and indirect organogenesis, multiplication through microrhizome induction, and propagation through protocorm-like bodies were frequently tried for the successful micropropagation of this genus.

Keywords: Axenic culture, Callogenesis, Callus, Explant, *Hedychium coronarium*, *H. flavum*, *H. forrestii*, *H. spicatum*, Hormones, *In vitro* propagation, Meristem, Micropropagation, MS medium, Organogenesis, Plant growth regulators, Protocorm, Regeneration, Somatic embryogenesis, Tissue culture, Totipotency.

INTRODUCTION

Micropropagation of medicinal plants has become significant in providing high-

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yielding elite genotypes for pharmaceutical purposes, as well as in producing high-quality plantlets for conservation [1]. Due to excess demand and injudicious harvesting, deforestation, climate change, pollution, urbanization, and natural calamities, several medicinal plants are in peril in their natural habitat [2, 3]. Variations in different biotic and abiotic environmental conditions severely limit the conventional method of propagation [4]. As a result, *in vitro* propagation methods may provide a better alternative and make it possible to rapidly multiply 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 to 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 [5, 6]. 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 to produce valuable phytoconstituents in greater quantities or with better properties, or both [7 - 18].

The *Hedychium* comprises approximately 100 species worldwide [19, 20]. Various species are used by traditional medicinal practitioners for treating asthma, gastric diseases, bronchitis, blood purification, anti-emetics, *etc* [21 - 25]. Furthermore, *Hedychium* species are extensively cultivated for their essential oils, and the aerial stems are a suitable raw material for manufacturing paper [26]. The essential oils and other phytoconstituents were extracted and characterized from these plants' leaves, flowers and rhizomes. It is well established that these compounds have numerous medicinal properties, including anticancer properties [24, 25, 27 - 35].

Hedychium species are rhizomatous perennial plants with pseudo stems, whereas rhizomes are true stems. Micropropagation of *Hedychium* species is quite infrequent, with only about 20 species studied. Direct organogenesis from axillary meristems was the most studied *in vitro* method, but multiplication through callus cultures and somatic embryogenesis were also studied in a few species. This chapter describes in detail the micropropagation methods used and the results of the medicinal herb *Hedychium*.

MICROPROPAGATION OF *HEDYCHIUM* SPECIES

Hedychium bousigonianum Pierre ex Gagnep

Hedychium bousigonianum is a narrowly distributed taxon native to Cambodia and Vietnam [19]. Micropropagation studies are comparatively rare in this species, with only somatic embryogenesis studies conducted.

Somatic Embryogenesis

In vitro production of *H. bousigonianum* through somatic embryogenesis and the effect of ethylene inhibitors like SA (salicylic acid) and $AgNO_3$ (silver nitrate) on the multiplication of primary somatic embryos (SE) and secondary somatic embryos (SSE) were studied in this species [36]. Seeds of *H. bousigonianum* were obtained from greenhouse-grown plants and subjected to scarification in 98% sulfuric acid (H_2SO_4) for 30 minutes. Afterward, they were rinsed three times for 5 minutes each with running tap water to eliminate any acid residues. Surface sterilization was performed under a laminar flow hood by dipping the seeds in 100% ethanol for 5 minutes with gentle shaking. Subsequently, the seeds were rinsed with sterile distilled water and transferred to sterilized beakers containing a 40% (v/v) sodium hypochlorite solution along with one drop of Tween 20 (Sigma, St. Louis, MO). The beakers were shaken for 20 minutes at 110 rpm. Following this, the seeds were rinsed three times with sterile distilled water and soaked overnight in sterile distilled water on a shaker at 110 rpm, at room temperature (22°C). The next day, the seeds underwent an additional three to four rinses with distilled sterile water before being transferred to 100 mm × 15 mm (diameter × depth) petri dishes. These petri dishes contained Murashige and Skoog (MS) basal medium supplemented with 20 g/L sucrose, 0.75 g/L $MgCl_2$, and 2 g/L Gelrite. Leafy explants were excised from 5–7 days old seedlings germinated *in vitro* using Murashige and Skoog (MS) basal medium [37] augmented with 2 g/l Gelrite, 0.75 g/l magnesium chloride ($MgCl_2$), and 20% sucrose. For callus initiation and proliferation (Table **1**) MS basal salts augmented with B_5 vitamins (Gamborg B_5) [38], 20% sucrose, myo-inositol (0.2 g/l), casein hydrolysate (1 g/l), thiamine (1 mg/L), $MgCl_2$ (0.75 g/l), Gelrite (2 g/l), 9.05 µM 2,4-Dichlorophenoxyacetic acid (2,4-D), and 4.6 µM of kinetin (Kn) (Sigma, St. Louis, MO) were used. All the media were sterilized by autoclaving at 121°C for 15 min. Calli were formed within 2 to 3 weeks after the inoculation of leaf explants on callus induction and proliferation medium. To promote the formation of somatic embryos, the fragile callus produced in the previous stage was shifted to a liquid media containing MS basal salts along with B_5 vitamins, 8.9 µM BA (benzyl adenine), 0.6 µM of TDZ (thidiazuron), 20% sucrose, myo-inositol (0.2 g/l), casein hydrolysate (1 g/l), and thiamine (1 mg/L) (Table **1**). Further analysis of the impact of different concentrations of silver nitrate ($AgNO_3$; 0, 10, 20, 30, 40, 50, 60 µM) and salicylic acid (SA; 0, 25, 50, 75, 100, 125, 150 µM) on the growth of the callus, development of the primary and secondary somatic embryo (SE and SSE) was performed. Higher concentrations of ethylene inhibitors reduced callus growth, but at concentrations of 30 to 50 µM $AgNO_3$ and 75 and 100 µM SA, there was an improvement in primary and secondary somatic embryo production. The study found that all concentrations of SA and $AgNO_3$ induced more SE and SSE formation than the control, despite a decrease in embryo

CHAPTER 7

The Green Treasure -*Jatropha curcas*

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Abstract: *Jatropha curcas* plants are a rich source of several natural components, the great majority of which are utilized for human health and the treatment of various ailments. *J. curcas* is a perennial plant in the family Euphorbiaceae that is gaining commercial significance due to its various industrial and medical uses. The purpose of this study was to improve the micropropagation conditions of *J. curcas* by using single node explants using the tissue culture technique.

Keywords: *Jatropha curcas*, *in vitro*, plant growth regulators, callus formation, single node.

INTRODUCTION

The Euphorbiaceae family includes the perennial shrub or small tree *Jatropha curcas* L. Common names for this plant include Barbados nut, fig nut, termite plant, caracas nut, black vomit nut, purge nut and physic nut. The plant grows well in tropical and subtropical settings, and its oil contains numerous therapeutic qualities [1]. The tree's quick growth, resilience, and ease of propagation allow it to thrive in a variety of precipitation patterns and adverse climatic conditions. Because *J. curcas* plants contain mechanisms that minimize dehydration and reduce leaf transpiration, they are able to withstand drought [2]. Stomatal closure limits gas exchanges, which causes the plant to lose leaf area, lowering photosynthesis and stomatal conductance in wet soil [3].

The plant's height ranges from three to five meters, and under optimal conditions, it can reach heights of 10 meters. The plant has articulated growth, a straight stem, and greenish-bronze branches with smooth wood [4]. Jatropha is a monoecious plant with an about 29:1 ratio of male to female flowers [5]. The seed kernel is composed of 57 to 63% fat and 22 to 27% protein [1]. It is possible to extract

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between 4 and 40 (%) of the viscous Caracas oil from its seeds [6]. It was found that the plant's lifespan is approximately 50 years [4].

It reproduces sexually through seeds and vegetatively through stem cuttings. Stem cuttings are typically preferred to seeds for plant propagation [1]. It was discovered that Jatropha propagated vegetatively produced few seeds. It was also discovered that vegetative propagation cannot produce plants with deep roots that can be easily uprooted [6]. Due to its numerous useful applications, multiple researches on Jatropha tissue culture and genetic manipulation have been done on a huge scale.

Biodiesel made from biomass is one of the most enticing technologies, as fatty acid methyl esters formed from vegetable oils are the best diesel replacement [7]. *Jatropha* species are rich producers of phytochemicals such as terpenes, cyclic peptides, alkaloids and lignans [4], in addition to their commercial worth.

This plant is of considerable therapeutic value since it contains components used to treat toothache and inflammation, as well as plant extracts for allergies, wound healing, and burns. Additionally, it is used to treat smallpox, leucoderma and leprosy. Its branches have been proven to be effective against HIV and cancer [8]. Jatropha is considered a non-edible biofuel source and is referred to as a biofuel source of the first generation.

It can be propagated by tissue culture procedures to alleviate the issue of lengthy cultivation and low yield [9]. A great number of plants can be cultivated from a tiny amount of endangered species' tissue. The meristematic tip culture is favored since this region is regarded to be virus-free. The physiology of the plant induced the plant's response to tissue culture.

The clonal propagation by tissue culture has the ability to yield high multiplication rates of homogenous genotypes, leading to short-term productivity improvements. As research proceeded, tissue culture mediums became more precisely defined, and several new tissue culture techniques were applied to *J. curcas*. These techniques have not yet been widely utilized for commercial operations on a broad scale. However, replication of high-oil-content *J. curcas* clones offers the opportunity for rapid establishment of high-yielding plantations. The propagation of plant regeneration through axillary buds is dependent on preexisting meristems. In this procedure, existing axillary buds are stimulated to develop into branches. This method takes advantage of the normal ontogenetic pathway of branch development by lateral (axillary) meristems. *In vitro* proliferation using meristem culture is also the most effective method for eliminating viruses [10].

This chapter focuses on the identification of the jatropha plant in terms of its physiological and chemical characteristics, as well as features of tissue culture for the *in vitro* reproduction of *J. curcas*.

PHYSICAL CHARACTERISTICS

J. curcas is a monoecious plant. Inflorescences form on the terminals of branches and contain both male and female flowers. *J. curcas* produces small greenish yellow flowers; the flowers are usually unisexual. Only a few male flowers are produced in each inflorescence. All flowers open at the same time, therefore, cross-pollination could occur between flowers from the same or other plants: twice a year in Egypt, the first in April and the second in December [11].

Though native to North America, the species has become almost pantropical in recent years due to its widespread use as a medical herb. Jatropha matures (over 8 years old) to a height of 7-13 meter, has spreading branches and stubby branches coated in a golden rufescent exudate. The leaves are green and thick, measuring 8.55 cm in length and roughly 5 cm in breadth, with a heart-shaped form and a long neck reaching up to 11 cm in length. Jatropha bears little greenish-yellow flowers and fruits that are green at first and turn yellow to brown as they develop. Typically, the fruit includes two to three oval black seeds; the seeds have kernels and shells; the kernels contain protein (22 to 28%) and high oil content (between 32 and 40%), depending on the growth circumstances and genotype [12].

The fruits measure around 2.5 cm in length and contain three black seeds; they attain full size roughly 90 days after pollination, divided into 30-45 days after pollination, yellow fruit stage (mature 45- 60 days) [13].

Plants are a rich source of several natural substances, the majority of which have been extensively utilized for human well-being and disease treatment. *J. curcas* is an adaptable, drought-tolerant perennial that is gaining economic relevance due to its numerous industrial uses and medicinal characteristics. *J. curcas* has been used traditionally to treat a variety of infections. Numerous physiologically active chemicals have been identified and described from all sections of this plant.

Medicinal herbs such as *J. curcas* have long been used to cure a variety of ailments, including bacterial and fungal infections. The name “jatropha” is derived from the Greek terms “jatros” (doctor) and “trophe” (meal), suggesting that it has medicinal qualities [14]. A drought-resistant shrub or tree that is found either wild or in semi-cultivated places across Central and South America, Africa, India, and Southeast Asia [15]. All parts of the Jatropha plant, including the leaves, seeds, bark, and other parts, have long been used in traditional medicine [16].

CHAPTER 8

Moringa oleifera Lam.: An Updated Review on Micropropagation and Pharmacological Properties

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Abstract: *Moringa oleifera* Lam. tree is considered a miracle tree due to its nutrient-rich profile. This plant has been widely cultivated throughout the world for its medicinal and nutritional benefits. Moringa plant contains a significant amount of various phytochemicals such as alkaloids, flavonoids, saponins, tannins, and phenolics that are responsible for their various medicinal, nutritional, and other applications. The use of moringa in pharmacological drugs not only increases cultivation but also increases the over-exploitation of this tree. As the conventional methods of moringa reproduction are not much effective, we require additional alternative strategies to multiply moringa plants. Micro-propagation is considered an effective method to produce a large number of transplants within a short time period. This chapter explores the micro-propagation approaches of *M. oleifera* together with its nutritional profile and biological activities.

Keywords: Antioxidants, Antidiabetic, Anticancer,Bioactive compounds, Flavonoids, *In vitro* propagation, *Moringa oleifera*, Micro-propagation, Miracle tree, Pharmacological activities.

INTRODUCTION

Moringa oleifera Lam. is a tropically grown extraordinarily medicinally miracle tree with a high commercial value. This plant also known as *Moringa pterygosperma* Gaertn., belongs to the angiospermic family Moringaceae. *M. oleifera* is very popular for its various nutritional benefits and pharmacological properties. The different morphological parts of this plant contain a variety of important phytochemicals which contribute to its medicinal uses. Phytochemical analysis of *M. oleifera* plant parts revealed the presence of saponins, flavonoids, alkaloids, tannins, glycosides, oil, carbohydrate, protein, polyphenols, reducing sugar, vitamins, resin, and carotenoids, *etc.* These bioactive compounds enable the

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plant to treat various ailments such as hepatoprotective, hypolipidemic, cardiovascular, hepatorenal, gastrointestinal, hypoglycemic, diuretic, and hematological disorders. The pharmacological attributes of *M. oleifera* include antidiabetic, anticholestrolmic, anti-inflammatory, antioxidant, antihypertensive, anticancer, antifertility, antiviral, antipyretic, antimicrobial, anti-atherosclerosis, anti-depression, anti-allergic, anti-rheumatoid and anti-ulcer properties.

M. oleifera is a locally available plant species. As it has been estimated that the global population will increase to 9.6 billion by 2050, we need to increase food production to 70% by 2050 [1]. To fulfill these future goals, we need to analyze other alternative food sources. *Moringa oleifera* has been considered as a 'Miracle tree' or 'Tree of Life' due to its vast nutrient and metabolite profile. Every part of this plant is nutritionally significant, such as leaves, flowers, fruits, roots, and seeds, which makes it peculiar to other plant species. As the moringa tree is full of nutrients, it can be a great food source for humans as well as for animals. *M. oleifera* plant has the potential to reduce malnutrition worldwide. The WHO (World Health Organization) recommended moringa as a complete food source to overcome starvation conditions [2].

M. oleifera plant has been used for a long time in traditional medicine. The world's oldest medicinal system 'Ayurveda' believes that this plant can prevent up to 300 diseases [3]. *M. oleifera* has been used for the treatment of diverse ailments such as diarrhea and dysentery (seeds), constipation, scurvy, convulsion and dropsy (leaf), jaundice, rheumatism, high blood pressure, beriberi and bronchitis (root bark), *etc* [4].

In the current research, phytomedicines are gaining more attention than conventional drugs. *M. oleifera,* currently being labelled as a 'super food', contains a long list of nutrients. Among the nutrients, proteins and lipids contribute a major part of moringa dry weight. *M. oleifera* leaf consists of 44% amino acid content, followed by flower (31%) and pod (30%) [5]. Many studies have also been published on the potential of moringa seed oil as edible oil. *M. oleifera* seeds consist of a high value of oil (33.69%), fibers (5.10%), proteins (37.78%), and ash (3.69%) [6]. Oil content is mainly represented by palmitic acid and oleic acid fatty acids.

As moringa leaves consist of high nutritional value, Foidl *et al*. [7] developed a growing technique for moringa to produce more leaf mass. They recommended high-density growing to reduce cost and increase yield. It is reported that 580 metric tonnes of fresh leaves and stems per hectare per year were harvested from a 4-year-old moringa plant *via* this technique. Plant tissue culture of *M. oleifera* through plant cells, and tissues are the best alternative to the conventional ways of

propagation to produce a high number of transplants. Plant regeneration of *M. oleifera* was performed by using direct and indirect pathways.

APPLICATIONS OF *MORINGA OLEIFERA*

M. oleifera is a multipurpose tree with nutritional and medicinal health benefits. The scientific efforts of various researchers contribute to understanding the diverse applications of *M. oleifera* as depicted in Fig. (**1**).

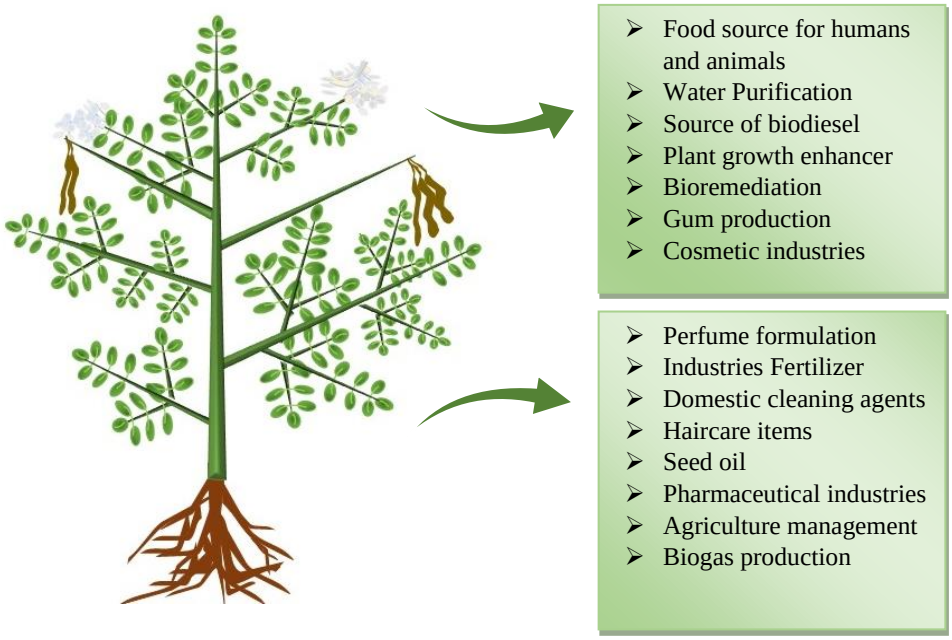

Fig. (1). Multipurpose tree *Moringa oleifera* Lam. with its diverse applications.

- **Food source**: *M. oleifera* is a highly nutritious tree. The most nutrient-dense part is the leaf. *M. oleifera* leaves contain many times higher nutrients compared to other vegetables such as 25 times higher iron than spinach, 17 times higher calcium than milk, 4 times higher protein than eggs, 7 times higher vitamin C than oranges, 10 times more vitamin A than carrots and 15 times higher potassium than bananas [8].
- Its leaves are generally used as animal feed in many regions of the world. Chizonda [1] carried out a study to reveal the potential of moringa as a dairy feed. The effects were observed of *M. oleifera* based diet in rumen fermentation by comparing it with alfalfa. It is found that the moringa plant increases digestibility and provides high protein feed. *M. oleifera* consumption also results in lower production of greenhouse gas (methane) than alfalfa. *M. oleifera* leaves

CHAPTER 9

Micropropagation and Biotechnological Interventions in *Oldenlandia umbellata* L.

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Abstract: *Oldenlandia umbellata* is an important medicinal herb distributed in the tropics used in the formulation to treat asthma, bronchitis, bronchial catarrh, snake bite, and many infectious diseases. The mature roots of *O. umbellata* are also known as a source of the natural dye anthraquinone (AQ), the second largest group of textile dye. However, extraction of the dye contained in the roots of this plant may pose a threat to its survival in its natural habitat. This chapter explores the scope and relevance of micropropagation of medicinally and economically significant *O. umbellata*, thereby saving this species in the wild and scaling up dye production through *in vitro* means. *In vitro* propagation efforts of this herb were made through axillary bud proliferation using benzyl adenine and by adding an auxin transport inhibitor, quercetin. A somatic embryo-based propagation system was also established through an *in vitro* starvation method. Based on available methods, *O. umbellata* can be efficiently propagated and conserved superior germplasm by applying the most suitable *in vitro* propagation methods.

Keywords: Anthraquinones (AQ), Auxin transport inhibitors, Benzyl Adenine (BA), Naphthalene Acetic Acid (NAA), 2,4-Dichlorophenoxyacetic acid (2,4-D), Indole-3-Butyric Acid (IBA), Indole-3-Acetic-Acid (IAA), Coconut Milk (CM), Micropropagation, *Oldenlandia umbellata*, Somatic embryogenesis.

INTRODUCTION

Human civilization began with using plants to formulate food, medicine, and cosmetics and was later used in many other areas, including textile dying and paintings. Many plants that we see around us have natural dyes within them that can be sustainably utilized. One such dye-yielding plant is *Oldenlandia umbellata* L., a herbaceous member of the family Rubiaceae.

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The genus *Oldenlandia* comprises about 300 species, with several endemic and threatened [1, 2]. This genus has also been called *Hedyotis* L. by Dassow in 1757, and it still appears under this name in some botanical literature [3]. The genus mainly includes shrubs, under shrubs, or herbs that are dichotomously branched. The leaves are opposite, in rare cases ternately whorled. The flowers are white to pink, sometimes blue, with a funnel-shaped corolla.

This genus is highly commended for its medicinal uses in Indian folk and Sidha treatment systems. The medicinal properties of different species of *Oldenlandia* were well studied, and many of them are rich sources of cyclo-peptides [4]. Different species of *Oldenlandia* have wide pharmacological activities such as uterotonic, anti-HIV, anti-neurotensive, and cytotoxic properties [5]. Over 20 species from the *Hedyotis–Oldenlandia* complexes, such as *O. biflora, O. corymbosa,* and *O. diffusa*, are well-known as a folk medicine for the treatment of cancers, infections, remittent and bilious fever, and other diseases in many Asian countries [6]. Some species of the genus *Oldenlandia* have shown a remarkable anticancer effect.

Oldenlandia umbellata L. is a small dichotomously branched biennial or perennial herbaceous plant with a quadrangular stem and sessile, opposite rough leaves [7] (Fig. **1A**). *O. umbellata* has a chromosome number 2n=36 in the somatic cells [8]. The plant is known to the general public as Indian madder or chay root (chayaveru). This Indian herb grows wild in sandy or loamy soils of coastal areas and is found in plenty along the southern coast of peninsular India, Pakistan, Ceylon, and West Tropical Africa [9]. *O. umbellata* yields red pigment from its roots and has been used in diverse applications since prehistoric times. The root bark, preferably of a two-year-old plant, when used with a mordant, will confer red colour to calico, wool, and silk fabrics [10]. The root extract of *O. umbellata* contains different constituents of AQ, such as alizarin and purpurin. AQ comes under the quinone group of phenolics. They are organic aromatic compounds, formula $C_{14}H_8O_2$, also known as anthracenedione or dioxoanthracene. AQs are valued as therapeutic agents due to their antimicrobial, analgesic, hypotensive, anti-inflammatory, anti-genotoxic, antitumor, and immune-enhancing effects.

In the past, British India witnessed the cultivation of Indian Madder as a dye-yielding crop, especially in the southern parts of the peninsula. It was through a letter addressed to a friend by James Anderson, a Scottish physician and botanist who structured two botanical gardens in Madras, that the fame of dye-yielding Indian madder reached the Western world [11]. In his letter, he noted, “It grows everywhere here (South India) as a small weed; but it is only by particular culture the roots become possessed of the beautiful and permanent red dye, the seeds of which only are preserved for the crop”. He also mentioned the possibility of

cultivating *O. umbellata* in the West Indies, since soil conditions in the Coromandel and West Indies are similar. He concluded the letter with a brief description of methods of cultivation of *O. umbellata* implemented in Northern Coromandel, including Madras.

Table 1. *In vitro* studies conducted in *O. umbellata*.

Explant type	Media	Outcome	References
Leaf, fruits, stem, axillary buds	½ MS+0.2mg/L BA +0.1%ascorbic acid + 1.5%sucrose	Somatic embryogenesis and whole plant regeneration, production of embryoids.	[21]
Young leaves, shoot apices, stems	MS+0.2 mg/L NAA, 0.5 mg/L BA, and 0.1% coconut milk	Organogenesis and somatic embryogenesis.	[22]
Nodal segments	MS+1mg/L BA,0.5 mg/L IAA.	Multiple shoot induction and multiplication, *in vitro* and *ex vitro* rooting.	[23]
Internodes	MS+2.5 µM 2,4-D,2.5µm NAA	Somatic embryogenesis and whole plant regeneration.	[24]
Nodal segments Young leaves, shoot apices, Nodal segments	MS+5µM BA + 5 µM Quercetin MS+0.7 mg/L NAA + 1.5 mg/L BA with 0.4% CM MS+1mg/L BA, 0.5 mg/L IAA.	*In vitro* shooting *In vitro* flowering Foliar micromorphological response of *in vitro* regenerated and field transferred *O. umbellata*.	[26 - 28]

MEDICINAL IMPORTANCE OF *O. UMBELLATA*

Since prehistoric times, Chayroot has been a part of India's traditional medical practices. Leaves and roots are good expectorants for treating asthma, bronchitis, and bronchial catarrh [12]. Pharmacological studies proved antitussive [13], antibacterial [14], hepatoprotective and antioxidant activities [15]. The decoction prepared from its roots and leaves is used externally as a wash for toxic bites and ulcers [14, 12]. *O. umbellata* is valued in Chinese systems of medicine due to various pharmacological properties like styptic, expectorant, and cholagogue action [15, 16]. The ethnic community in China uses *O. umbellata*-based extracts as first aid to snake bites, particularly for pit vipers. *O. umbellata* has also been employed to treat sores and carbuncles on the skin, appendicitis, sore throats, urinary tract infections, and hepatitis.

CHAPTER 10

Tuberoid Orchids: Micropropagation for Biomedical Applications

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Abstract: The article presents the developed protocol for the propagation of tuberous orchids and their organs. The protocol allows for the cultivation of materials for reintroduction, restoration of disturbed populations and biomedical research. A phytochemical analysis of this plant group was conducted for the first time, confirming their medicinal properties. Micropropagation was employed to achieve this objective. The study focused on nine species of tuberous orchids from the Orchidaceae family: *Dactylorhiza traunsteineri, D. maculata, D. fuchsii, D. incarnata, D. urvilleana, D. baltica, Gymnadenia conopsea, G. conopsea f. gigantea* and *Orchis militaris*. The results demonstrated that these species contain chemical substances with potential physiological activities. For example, squalene exhibited positive effects such as antifungal, anticancer, antibacterial, antioxidant, and others. Additionally, other identified chemical substances demonstrated antiproliferative and proapoptotic activities against colon cancer, as well as antibacterial, anti-inflammatory, antioxidant, antitumoral, and antifungal properties.

Keywords: Biologically active substances, Culture medium, *Dactylorhiza*, *Gymnadenia*, *In vitro*, Micropropagation, *Orchis*, Orchid, Pyrolysis-gas chromatography/mass spectrometry, Secondary metabolites, Tissue culture, Tuberoid.

INTRODUCTION

The earliest mention of the use of orchids in medicine dates back to the 28th century BC. Shen-nun described *Bletilla striata* and a species of *Dendrobium* in the Materia Medica. In India, certain orchids such as *Eulophia campestris*, *Orchis latifolia*, and *Vanda roxburgii* have attracted the attention of the scientific community due to their medicinal properties [1, 2]. The presence of compounds such as alkaloids and flavonoids suggests their healing properties. References provide information on the use of tuberous orchids in traditional medicine, where

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preparations from these plants exhibit anti-inflammatory, soothing, and emollient effects [3]. They are employed for the treatment of acute and chronic diseases of the upper respiratory tract, hyperacid gastritis, peptic ulcers of the stomach and duodenum, as well as gastroenteritis and colitis. Mucilage derived from orchids is also used in cases of acute poisoning [4].

Insufficient research has been conducted on the medicinal properties of orchids, and the specific biologically active components responsible for these effects remain unknown [5]. The biosynthesis, transport, and accumulation of secondary metabolites in orchids are poorly studied. According to available literature, tuberoids are known to contain mucus (50%), starch (31%), dextrin (13%), sugars (11%), protein substances (5%), mineral salts, and bitter substances [6, 7].

Tuberoid orchids are not a taxonomic group; rather, they encompass species that possess tuberoids as underground structures. A tuberoid is a specific type of underground organ that represents a thickened aerial part of the stem. Its primary function is to store water and nutrients, serving as an adaptation to seasonal climates with cold winters [8]. Currently, the term "root-stem tuberoid," proposed by Dressler [9], is widely used to describe this structure. There are 85 species from 21 genera that grow in the territory of European Russia and Eastern Europe [10].

Currently, tuberoid orchids are classified as rare and protected species listed under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) [11, 12]. They are listed in Appendices I and II, which makes their use in research challenging. This is primarily due to the complex bioecological characteristics exhibited by this group of species, including saprotrophic development during the initial stages of ontogenesis, a short life cycle, mycosymbiotrophism, anthropogenic alteration of natural habitats, and poaching.

A significant amount of experience has been accumulated in the *in vitro* cultivation of temperate zone orchids from seeds. The formation of protocorms, primary shoots, and adventitious roots has been well studied for some species [13 - 18]. Special emphasis is placed on the propagation of tuberoid orchids. In natural populations, a limited ability for vegetative reproduction is observed [19, 20]. However, active vegetative reproduction at the protocorm stage has been observed under laboratory conditions *in vitro* [17, 21 - 23].

Limited data are available due to the challenges associated with obtaining materials for study. Orchid cultivation technologies have primarily focused on ornamental and flowering species, with tuberoid orchids receiving less attention from biotechnologists for a long time [24]. The exploration of the medicinal potential of this plant group necessitates the investigation of the quantitative and

qualitative composition of secondary metabolites. In this study, we conducted a biochemical screening to analyze the qualitative composition of metabolites in tuberoid orchids and examined their properties.

MATERIALS AND METHODS

Plant material

Dactylorhiza traunsteineri, D. maculata, D. fuchsii, D. incarnata, D. urvilleana, D. baltica, Gymnadenia conopsea, G. conopsea f. gigantea and *Orchis militaris* were the subjects of the study Table **1**. These plants were selected due to their wide distribution and their possible practical application. All these species grow on the territory of European Russia. The seeds were collected in the Botanic Garden of Lobachevsky State University of Nizhny Novgorod in 2015.

Table 1. Classification of studied orchid species.

Subfamily	Tribe	Genus	Species
Orchidoideae	Orchideae	*Dactylorhiza* Neck. ex. Nevski	*Dactylorhiza baltica* (Klinge) Orlova
			Dactylorhiza incarnata (L.) Soo
			Dactylorhiza fuchsii (Druse) Soo
			Dactylorhiza maculata (L.) Soo
			Dactylorhiza traunsteineri (Saut.exRchb.) Soo
			Dactylorhiza urvilleana (Steud.) H. Baumann & Kunkele
		Gymnadenia R. Br.	*Gymnadenia conopsea* (L.) R. Br.
			Gymnadenia conopsea forma *gigantea* (L.) R. Br.
		Orchis L.	*Orchis militaris* L.

According to the modern classification of the family, experimental species belong to 1 subfamily, 1 tribe and 3 genera.

Dactylorhiza baltica (Klinge) Orlova

Plant height: 30-60 cm, with tubers divided into four parts or deeply double. The stems are straight, thick, and hollow on the inside. The leaves are oblong-lanceolate and spotted. The length of the lower leaves ranges from 9 to 20 cm, with a width of 2 to 3.2 cm. The flowers are purple (Fig. **1a**). Regarding the perianth features, the middle outer leaflet and two lateral inner leaflets are curved into a helmet shape, while the lateral outer leaflets are also curved.

CHAPTER 11

Current Status of Micropropagation of *Operculina turpethum* (L.) Silva Manso – An Endangered Medicinal Plant

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Abstract: The use of cutting-edge biotechnological methods such as *in vitro* propagation enables the large-scale production of disease-free plant material, rapid cloning, and conservation of the elite genotype within a very short period. Additionally, the technique has enormous potential for the production of pharmacologically significant secondary metabolites and plant-based medicines of high quality. *Operculina turpethum* (L.) Silva Manso is an important medicinal plant of the family Convolvulaceae and is used to treat several health ailments. Overexploitation and inadequate conservation strategies have put the plant on the verge of extinction. This chapter provides a concise overview of the current status of the endangered medicinal plant *Operculina turpethum* with special attention given to the *in vitro* propagation and conservation of the immense medicinal plant.

Keywords: Apical bud, benzyl amino purine, endangered, *in vitro* propagation, kinetin, medicinal plant, micropropagation, nodal explant, *Operculina*, rooting, somatic embryogenesis, Trivrit, turpethein.

INTRODUCTION

The ever-growing herb-based pharmaceutical sector has exerted a significant strain on raw materials, the bulk of which are derived from plants that grow in forests or other natural habitats. While developed technologies are unused and plant cultivation is no longer practiced, the Indian drug and pharmaceutical industry continue to obtain nearly 90% of its supplies from wild collections, and

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more than 70% of plant collections entail destructive collection because of the use of plant components such as roots, bark, wood, and complete plants.

The increased demand for pharmaceutical industries is being fulfilled by nature. As plant species aren't effectively conserved, their germplasm supply is decreasing. Many species on the endangered list or on the brink of extinction in the wild need prompt conservation and large-scale production to rescue them from extinction, conserve their germplasm, and fulfill the pharmaceutical industry's enormous demand, which is vital for human health. More than 90 percent of medicinal plants in India are in danger of extinction as a result of unsustainable and excessive collection, usage, overexploitation, and untrained harvesting practices [1]. In the years 1987-1990, the Red Data Book of Indian Plants designated 602 species of vascular plants as threatened; this number rose to 1,255 in 2003 [2] and 2,152 in 2020 (IUCN 2020). Lists for human and veterinary use include 457 of 2,152 species. Among these, 73 are categorized as threatened (CR, EN, VU), 8 (Near Threatened), and 1 (Data Deficient) is included in this category, whereas 366 are listed under the Least Concerned category. The natural habitat for many herbs and trees is rapidly dwindling due to the rapidly growing global population, increasing anthropogenic activities, rapidly diminishing natural ecosystems, *etc.* Climate change and ocean acidification have contributed to the unsustainable exploitation of the Earth's biological variety, which has been worsened by other human-induced environmental consequences [3]. Every year, billions of dollars are spent to restore biodiversity using numerous methods. There has been no change in the natural habitat of these plant species, and the number of species on the brink of extinction has increased. Managing the traditional medicinal plant resources, therefore, has become a major challenge. Many medicinal plants do not generate seed or their seeds are too small to sprout in the soil, making in-situ conservation ineffective for endangered species. Even seed-grown plants vary widely in growth, habit, and productivity and may be eliminated owing to poor commercial quality. Most plants cannot be reproduced *via* cuttings or grafting, limiting cultivar replication. Many vegetatively produced plants contain systemic bacteria, fungus, and viruses, which affect quality and appearance. It is possible to apply *ex situ* techniques as a complement to *in situ* conservation measures, and in certain instances, these techniques may be the only option available [4, 5]. Therefore, the conservation of medicinal plants may be accomplished *ex situ*, in an environment outside of their native habitat. This can be done by cultivating and storing plants *via* the long-term storage of plant propagules in plant tissue culture repositories [3].

OPERCULINA TURPETHUM (L.) SILVA MANSO

Operculina turpethum (L.) Silva Manso is commonly known as Indian Jalap or Trivrit/Turpeth. The large perennial climber belongs to the family Convolvulaceae. The plant is found throughout tropical dry and moist deciduous regions in central and peninsular India even at higher altitudes of 900m. It is a herb that is found in Australia (Northern Territory, Queensland); Africa (Kenya, Tanzania, Mozambique, Zimbabwe; Western Indian Ocean: Madagascar, Mauritius, Reunion); Pacific (Northwestern Pacific: Micronesia); Asia-Tropical (India, Nepal, Pakistan, Sri Lanka, Indochina, Myanmar, Thailand, Indonesia, Malaysia, Papua New Guinea, Philippines); and Asia-Temperate (China). There are two types of Turpeth, commonly known as Shweta or White Turpeth and Krishna or Black Turpeth. According to Ayurveda, the plant has light (laghu), sharp (tikshan) and dry (ruksha) properties. It is pungent (katu) and bitter (tikta) in taste and hot (ushan) in potency [6]. As per an estimation in 2015-2016, *O. turpethum* is one of the highly consumed medicinal herbs in the Indian domestic herbal industry with an annual consumption of about 670 metric tonnes. Due to the wide applicability in the pharmaceutical industry, the plant is exported to other countries like the United States, Japan, Netherlands, Sri Lanka, and Singapore with an export value of 2.4 million USD which confirms the high demand of the plant in the market. Aside from its medicinal uses, the plant's seeds have been described as a potential source of commercial gum. However, over exploitation of the root/root bark over the other parts of the plant has resulted in the depletion of the plant's genetic resources in the wild, causing it to be classified as Near Threatened, which leads to a lack of available plant material and adulteration. *In vitro* multiplication is a viable option for large propagation of medicinal plant species where traditional approaches are limited.

Phytoconstituents of the Plant

The plant contains a variety of secondary metabolites which include phenolics, flavonoids, glycosides, saponins, steroids, coumarins, and carbohydrates. The aerial part of the plant contains turpethosides A, B glycosidal resin, and glycoside acids *i.e*., turpethic acids A-C [7]. Oleandrin, a cardiotonic agent with anti-inflammatory properties, is the active ingredient of the leaf. The bark, root, and seed include cardio-active glycosides, neriodorein, and karabin, which have anti-inflammatory, analgesic, and stimulating properties [8]. The root contains Stigma 5, 22 dien 3 O-β -D-glucopyranoside, β-sitosterol, scopoletin, betulin, cyclo-artenol, lanosta-5-ene, coumarin, acrylamide 3- (4-hydroxy-phenyl) -N-[2-(4-hydroxy-phenyl)-ethyl], salicylic acid, four dammarane-type triterpenoid saponins (operculinosides A, B, C, D), steroidal esters [7, 9]. A glycoside analog of jalapine and convolvulin, “turpethein” (turpeth resin) is also found in the root

CHAPTER 12

Recent Advances in Mexican Coriander (*Eryngium foetidum* L.) *In Vitro* Propagation

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Abstract: *Eryngium foetidum* L. is an important medicinal and aromatic plant of the family Apiaceae. The plant is extensively used in traditional medicine and for culinary purposes. The essential oil of the plant has very high economic value in both national and international markets due to its application in the pharmaceutical, cosmetics, and perfumery industries. The plant is generally propagated through seeds. However, due to low seed viability, the plant is restricted to certain regions, which in turn hinders the commercial application of the medicinal plant. Therefore, quick and mass multiplication of the plant is needed, which may be accomplished by micro-propagation. This is necessary in order to satisfy the ever-increasing demand of the pharmaceutical and cosmetic industries. In this chapter, a variety of methods for micropropagation have been explained, each of which utilises a different component of the plant as an explant.

Keywords: *Eryngium foetidum*, *in vitro* propagation, kinetin, micropropagation, nodal explant, rooting, somatic embryogenesis, adjuvant.

INTRODUCTION

There are around 228 plant species belonging to the genus *Eryngium* of the family Apiaceae. The species *Eryngium foetidum* L. is cultivated and domesticated worldwide and extensively used for therapeutic and culinary purposes and in the perfumery and cosmetic industries. The plant is commonly known as spiny coriander, Mexican coriander, spirit weed, cilantro, ban dhania, *etc.* The biennial tropical herb is used as a substitute condiment for coriander because of its similar pungent smell. The plant is native to tropical regions of the Americas and the

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West Indies, where it has a history of usage in both medicinal and culinary contexts. It has been naturalized and is often grown in South Asia, the Pacific islands, Tropical Africa, and the warmer southern sections of Europe. It is widely distributed in the Brazilian territory, with occurrences in Acre, Amazonas, Amapá, Pará, Rondônia, and Roraima, with a phytogeographic domain in the Amazon. Propagation of the species occurs through the seeds or reuse of clumps, which can be transplanted into the soil.

CHEMICAL PROFILE

The plant contains several secondary metabolites such as phenols, tannins, flavonoids, saponins, ascorbic acid, and terpenoids. The aerial parts of the herb are a rich source of calcium, iron, riboflavin, carotene, vitamins A, B, and C, and essential oils. The fresh leaves contain over 85% moisture, 3.3% protein, 0.6% fat, 6.5% carbohydrates, 1.7% ash, 0.06% phosphorus, and 0.02% iron. (E)-2-dodecenal is reported to be the major constituent of leaf essential oil which is responsible for the unique flavour and aroma of this herb. Its concentration varies with regard to different geographical locations such as in India (45.9%), Vietnam (45.5%), Malaysia (59.7%), Bangladesh (37.4%), the Venezuelan Andes (27.5%), South Vietnam (58–67%), Western Nepal (58.1%) and Sao Tome e Principe (15.9–37.5%). Phytochemical screening of the leaves indicated the presence of unbound triterpenoids, α-cholesterol, brassicasterol, campesterol, and stigmasterol being the main components totalling 95%, and clerosterol, β-sitosterol, δ-5-avenasterol, δ-(5)-24-stigmastadienol and δ-7-avenasterol as the remainder. Seeds of *E. foetidum* yielded 0.2% of essential oil in which carotol (19.3%) was identified as the major component.

PHARMACOLOGICAL ACTIVITIES

There are several studies on the pharmacological application of *Eryngium foetidum*. Borah *et al.* [1] and Lingaraju *et al.* [2] reported the antifungal activity of *E. foetidum* leaf extract against *Candida albicans, C. guilliermondi*, and *Cryptococcus neoformans*. The stigmasterol-rich leaf extract showed topical anti-inflammatory activity on chronic and acute inflammation in animal models [3]. Ethanol extract of *E. foetidum* leaf inhibited the elevation of interleukin 6 (IL-6), tumor necrosis factor-alpha (TNF-α), inducible nitric oxide synthase (iNOS), and cyclooxygenase (COX-2), together with their cognate messenger ribonucleic acid (mRNAs) in a dose-dependent manner. The methanol, chloroform and aqueous extract of *E. foetidum* leaves significantly inhibited the growth of gram-positive and gram-negative bacteria. Similarly, the suspension of blanched leaf resulted in 100% growth inhibition of *Staphylococcus aureus* and *Bacillus subtilis.* Again,

the ZnO nanoparticle prepared using *E. foetidum* leaf extract showed broad spectrum antibacterial activity. Eryngial, the major compound of *E. foetidum,* is reported to exhibit anthelminthic activity against the infective larvae of *S. stercoralis* and is significantly more effective than the standard drug, Ivermectin [4].

USE IN TRADITIONAL MEDICINE

The herb is used to cure gynaecological and gastrointestinal conditions such as flatulence, diarrhoea, indigestion, and stomach discomfort in South American nations like Peru, Colombia, and Ecuador. It is suggested to drink the plant's tea to cure female reproductive issues, encourage menstruation, ease cramps, treat infertility, and ease childbirth, and it is said to have aphrodisiac properties [5]. In four Amazonian riverbank settlements, *E. foetidum* was used as tea and syrup to treat flu, according to research by Vásquez *et al.* [6]. The entire plant is used as an antiscorbutic, antirheumatic, antiseptic, and to treat headaches, nausea, vomiting, and hemorrhage in Colombia. Another application is for bath preparation and for the treatment of gonorrhea and smallpox [7].

MICROPROPAGATION OF *ERYNGIUM FOETIDUM*

Plant Regeneration *via* Direct Organogenesis

Martin [8] established a plant regeneration protocol of *Eryngium foetidum*, using leaf, stem, disc, and root explant derived from *in vitro* grown shoots. He used MS (Murashige and Skoog's) basal media with or without sucrose and MS micro and macro nutrients without any organic compound. The MS basal medium with sucrose exhibits shoot initiation within 15 days of inoculation whereas shoot initiation was observed after 23 days for explant cultured in MS basal media without sucrose and MS micro and macro nutrient media. However, no significant difference was observed in the number of shoots and roots developed on the different types of media. The stem and disc explant produced 4 shoots and 2.6 roots and the leaf and root explant formed 1.5 shoots, 7.5 roots, 2.5 shoots, and 2.0 roots respectively. The well-developed plantlets planted on small pots containing sand and soil in a 1:1 ratio with 100% survival rate for plants grown in MS basal medium without sucrose and in MS micro and macro nutrient containing media. The plantlets developed in MS basal media with sucrose exhibited 80% survival upon field transfer [8].

Another study on *in vitro* regeneration of *E. foetidum* from leaf explant was reported by Gayatri *et al*. [9]. They used matured leaves as explant, which were

CHAPTER 13

A Review of Micropropagation of *Allium sativum* L. (Family: Alliaceae)

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Abstract: Garlic *(Allium sativum* L.) is the most often used medicinal plant and the second most commonly used *Allium* species after onion. It belongs to the Alliaceae family. Garlic originated in Central Asia and is currently cultivated all over the world. Garlic is rich in bioactive components and is used in various medicinal uses. Garlic has a greater concentration of sulfur-containing compounds, which contribute to its pungent odour. The major phytoconstituents of garlic are alliin, allicin, ajeones (oil-soluble organosulfur compounds); water-soluble organosulfur compounds such as S-allyl cysteine (SAC), metabolites allyl mercaptan (AM), allyl methyl sulphide (AMS), and S-allyl-mercapto cysteine (SAMC). Due to its bioactive components, garlic has various pharmacological properties, including anticancer, antidiabetic, anti-inflammatory, antioxidant, and antibacterial action. Garlic micropropagation is feasible due to its widespread use and robust pharmacological activity. Micropropagation of garlic, which includes meristem culture or shoot tip culture, is reported to have various advantages, including the ability to create disease-free plant material, develop a higher number of desired plants, enhance the bioactive of garlic, and improve crop quality. This chapter briefly summarizes garlic's bioactive components, their pharmacological actions, the role of micropropagation in garlic, and its application.

Keywords: *Allium sativum*, garlic, bioactive, organosulfur compounds, anticancer, antidiabetic, micropropagation, meristem culture, and shoot tip culture.

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INTRODUCTION

Garlic *(Allium sativum* L.) is the most often used medicinal plant and the second most commonly used *Allium* species after onion. It belongs to the Alliaceae family. Garlic originated in Central Asia and is currently cultivated all over the world. Garlic is rich in bioactive components and is used in various medicinal uses [1, 2]. Garlic has a greater concentration of sulfur-containing compounds, which is responsible for charity its pungent odour. The major phytoconstituents of garlic are alliin, allicin, and ajeones (oil-soluble organosulfur compounds). The water-soluble organosulfur compounds such as S-allyl cysteine (SAC), metabolites allyl mercaptan (AM), allyl methyl sulphide (AMS), and S-ally--mercapto cysteine (SAMC) are also found in garlic. Due to its bioactive components, garlic has various pharmacological properties. It includes anti-cancer, antidiabetic, anti-inflammatory, antioxidant, and antibacterial action. Garlic micropropagation is feasible due to its widespread use and robust pharmacological activity. Micropropagation of garlic, which includes meristem culture or shoot tip culture, is reported to have various advantages, including the ability to create disease-free plant material, develop a higher number of desired plants, enhance the bioactives of garlic, and improve crop quality. This chapter summarizes garlic's bioactive components, pharmacological actions, micropropagation in garlic, and its applications [3, 4].

In vitro plant propagation can be divided into two categories: (a) In terminal or axillary buds propagation, existing meristems are used. The techniques used are meristem culture, shoot-tip culture, and node (single or multiple) cultures. (b) Explants derived from somatic tissues are used for propagation, forming adventitious somatic embryos or adventitious shoots. Embryos/adventitious shoots can arise directly from the cut explant tissues without forming callus (direct embryogenesis or direct organogenesis). Instead, embryos or shoots develop into a callus (indirect embryogenesis or indirect organogenesis) [5].

A. sativum is a good source of pharmacologically active molecules. Due to the presence of many bioactive components, *A. sativum* possesses many pharmacological activities like antifungal activity, antibacterial activity, anti-protozoal activity, anti-cancer activity, antidiabetic activity, anti-inflammatory, antioxidant activity, antiviral activity, anti-obesity, anti-atherosclerotic, neuroprotective, and immunomodulatory properties [1, 6, 7]. Therefore, it treats colds, whooping cough, and earache, stomach, and lung disorders, prevents cardiovascular disease, and helps digestion [8, 9].

BIOACTIVE COMPONENTS OF *ALLIUM SATIVUM* L.

Many elements, especially macronutrients, are found in equal amounts in *Allium* vegetables; however, garlic contains several minerals, notably selenium [10]. *A. sativum* exerts pharmacological activities due to their bioactive components, *viz*., alkaloids, flavonoids, glycosides, phenols, tannins, steroids, terpenoids (mono-, di-, tri-, and sesquiterpenes), and saponins. Garlic contains 28% carbohydrates, 2%, and 1.2% proteins and amino acids, 1.5% fiber, fatty acids, more than 33 sulfur-containing compounds (2.3%), and 65% water. The significant phytoconstituents of garlic include alliin, thiosulfinates (allicin), ajoenes (E-ajoene, Z-ajoene), sulphides: diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), allyl methyl sulfide (AMS), vinyl dithiins (2-vinyl-(4H) -1,3-dithiin, 3-vinyl-(4H)-1,2-dithiin), N-acetylcysteine (NAC), S-allyl cysteine (SAC), metabolites allyl mercaptan (AM), allyl methyl sulfide (AMS) and S-allyl mercapto cysteine (SAMC). Alliin, allicin, and ajoenes are oil-soluble organosulfur compounds, whereas SAC, SAMC, AM, and AMS are water-soluble organosulfur compounds of garlic [1, 7]. The polysaccharides content of garlic comprises fructose 85%, glucose 14%, and galactose 1% [11].

NEED FOR TISSUE CULTURE IN GARLIC

It is feasible to increase the callus formation of garlic to enhance its propagation coefficient. In the development of garlic calluses, genotypes, types of explants, and their interactions all had a substantial influence. The study revealed notable variations in callus production among garlic cultivars. In an analysis for phytohormone content, auxins (methyl indole-3-acetic acetate and indole acetic acid), cytokinins (di-hydro zeatin and trans-zeatin (ZT)), abscisic acid (ABA), gibberellins (GA_3), jasmonic acid (JA), dihydro jasmonic acid, and jasmonoyl-L-isoleucine were significantly higher in the meristem tips than upper leaf parts. Therefore, high levels of natural jasmonic acid could be crucial for callus development. The best explants for inducing calluses were stem tips and interior leaves [12].

A. sativum is grown through vegetative propagation, which can result in viral accumulation, degradation of germplasm, a lower propagation coefficient, and high production cost. The fastest way to multiply virus-free garlic and improve its germplasm is through micropropagation; as many as eleven adventitious buds could be created from a single explant by adjusting the kind and plant growth hormone concentration. After the culture method and induction settings were optimized, the bulblets propagation coefficient *in vitro* could achieve 10.64 per explant. A specific viral eradication effect of garlic tissue culture was also demonstrated using Enzyme-Linked Immunosorbent Assay (ELISA). This

CHAPTER 14

A Review of Micropropagation of *Glycyrrhiza glabra* L. (Licorice)

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Abstract: *Glycyrrhiza glabra* belongs to the family Fabaceae and is commonly called licorice. It is an important medicinal plant in Europe, China, and the Mediterranean. The plant's therapeutic value is also mentioned in Ayurveda and Siddha. Licorice is cultivated for commercial purposes in many parts of the world because of its economic value and demand. It is used as a flavoring agent in juices, candies, soft drinks, and beverages because of its characteristic taste and smell. In addition, it is regarded as a sweetener and thirst quencher. Licorice contains phytochemicals, and the most abundant compounds are glycyrrhizic acid, anethole, liquiritigenin, isoliquiritin, pinocembrin, and licoflavanone. The plant is a good source of antioxidants and exhibits anti-inflammatory, antimicrobial, antiviral, anti-diabetic, and anti-cancer activity. Even though it has many health-benefiting features, consuming high amounts of licorice can lead to hypertension, hypokalemia, and congestive heart failure. Due to its high demand, good medicinal value, and poorly developed cultivation strategy, researchers are focusing on different aspects of the *in vitro* propagation of the plant. Studies have revealed that micropropagation of licorice has improved the level of secondary metabolites and high antioxidant properties. Thus, this chapter focuses on the propagation method of licorice, primarily focusing on micropropagation. Moreover, it also highlights the phytochemistry and important pharmacological activity of *Glycyrrhiza glabra.*

Keywords: *Glycyrrhiza glabra*, Micropropagation, Phytochemistry, Glycyrrhizic acid, Multiplication, Plant regeneration, Shoot induction.

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INTRODUCTION

Glycyrrhiza glabra L. is a perennial herb belonging to the family of Fabaceae and reaching a height of 1-1.5 m. The plant is commonly called licorice. It has woody stems from long cylindrical stolon; the plant has stoloniferous tap roots used for medicinal purposes in ancient Siddha medicine; they are fibrous, flexible, and bright yellow. The plant is considered native to Western Asia, Southern Europe, North Africa, Mediterranean region and is mainly cultivated in central Asia, north-western China, Iran, Turkey, Iraq, and parts of the United States [1]. The plant favorably grows in subtropical and warm temperate zones. The sweetness of licorice is due to a compound called Glycyrrhizic acid, structurally a saponin. The characteristic scent of licorice is due to anethole, a volatile unsaturated ether related to lignols.

Licorice is also a flavoring agent in the candy industry, soft drinks, beer breweries, liqueur and brandy manufacturing, and the tobacco industry [2]. Licorice is used as an ingredient in cough lozenges, syrups, and elixirs [3]. Licorice is used as naturopathic medicine to treat mouth and peptic ulcers. The plant's root extract possesses anti-inflammatory properties; it is also used as a demulcent to treat irritation of the mucous membranes in the mouth. The root has mild expectorant properties, thus used to treat cough. The plant extract is often used in elixirs, laxatives, and cough medicines as sweeteners [4]. Licorice is used for gastritis, cough, bronchitis, ulcers, inflammation, and epilepsy [5]. Compounds such as glycyrrhizin, dipotassium glycyrrhizinate, and glycyrrhetinic acid derived from *G. glabra* possess an anti-inflammatory effect by inhibiting High mobility group box 1(HMGB1), Toll Like Receptor 4 (TLR4), and Receptor for Advanced Glycation Endproducts (RAGE) receptors and up-regulating the function of essential cytokines, interleukins, and genes required in nuclear factor kappa B (NF-κB), HMGBI and Mitogen-activated protein kinase (MAPK) signaling pathway [6]. Hydroalcoholic extract of *G. glabra* has an inhibitory effect on the growth of *Leishmania major* promastigotes and amastigotes. As per reports, licorice significantly reduced the lymph node parasite burden and increased the level of Interferon-gamma (IFN-γ) and the ratio of IFN-γ/ Interleukin-4 (IL-4) in BALB/c mice infected with *Leishmania major* [7]. The flavonoid-rich extract of *G. glabra* improved the intestinal epithelial barrier integrity in human intestinal Caco-2 cells. Moreover, a significant decrease in the level of TNF-α, myeloperoxidase (MPO), and an increase in the level of IgA and tight junction proteins expression was observed in the colon of rats with 2,4,6-trinitrobenzene sulfonic acid (TNBS) induced ulcerative colitis [8].

Licorice has been used to treat flatulence, stomach ulcers, colic, hyperdipsia, and other digestive system diseases [9]. The root extract of *G. glabra* is used as an eye

drop to treat conjunctivitis in India. The root powder is mixed with honey, which is used to treat anemia. Moreover, the paste made from *Glycyrrhiza* and *Picrorhiza kurroa* with sugar water is used as cardiotonic [10]. Different categories of bioactive compounds are reported in *G. glabra*, which mainly include triterpenoid, coumarin, saponin, polysaccharides, flavonoid, isoprenoid substituted phenol, essential oils, sterols, simple sugars, glycosides, female estrogen hormones, resins, tannins, pectins, asparagines, gums, amino acids, volatile oils, fat, mineral salts, and starches. Most of the compounds are water soluble and constitute 40-50% of the total dry weight [11]. Glycyrrhizinic acids obtained from *G. glabra* are used to treat atopic dermatitis, pruritis, and cysts caused by skin parasites [12]. This plant contains many phytochemicals, including isoliquiritin, glycyrrhizin, glycyrrhizic acid, and glycyrrhizinic acid, which possess antispasmodic effect, anti-asthmatic, anti-inflammatory, anti-diabetic, anti-microbial, anti-cancer, and anti-atherogenic effect [11]

Glycyrrhizin, a potent bioactive of *G. glabra*, is found to have antiviral properties against hepatitis C virus (HCV), human immunodeficiency virus (HIV), coxsackievirus B3 (CVB3), H5N1 virus, coxsackievirus A16 (CVA16), duck hepatitis virus (DHV), herpes simplex virus type 1 (HSV1), enterovirus 71 (EV71) by interfering and hampering the virus activity *via* inhibition of gene expression, blocking the replication process, reducing HMGB1 binding to DNA and reducing adhesion force and stress components. It also improves host cell activity by hindering the degradation of IκB, the proliferation of T cells, and suppressing host cell apoptosis [13]. Isoflavones isolated from *G. glabra* were found to mitigate the oxidative stress in liver mitochondria by significantly reducing the di-hydroxy fumarate-induced mitochondrial lipid peroxidation induced by Fe^{3+}- ADP/NADH [14].

In addition, *G. glabra* exhibited nitric oxide (NO) generation inhibitory effect and DPPH radical scavenging activity. Furthermore, *G. glabra* aqueous extract inhibited the increased levels of inflammatory mediators such as NO and prostaglandin E2 (PGE2); moreover, it also induced the expression of Heme oxygenase-1 and inhibited inducible NO synthase, cyclooxygenase-2 and IFN-β, Tumor necrosis factor alpha (TNF-α), IL-6, IL-1β, NF-κB activation, Akt activation and STAT1 activation in Lipopolysaccharide-stimulated RAW264.7 macrophages thus inhibiting LPS-induced inflammation in RAW264.7 cells [15]. Glycyrrhetinic acid promotes hepatoprotective function by attenuating NF-κB activation in hepatocytes against chronic liver inflammation induced by TNF-α [16].

The protein extract of licorice has a potent anti-cancer activity. It significantly inhibited the growth and proliferation of human colon cancer and colon carcinoma

CHAPTER 15

Micropropagation of Vetiver Grass - A Review

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Abstract: Plants and plant materials have long been employed in traditional herbal remedies. In recent decades, natural resource demand has surged by 8–15% each year in Asia, North America, and Europe. Vetiver is a plant that produces essential oils and is widely used in perfumery, cosmetics, and herbal medicine. The utilisation of controlled conditions can help overcome traditional cultivation challenges and may be used to modify phenotypic variance in the concentration of bioactive chemicals. The goal is to improve extract potency, minimise toxin levels, and improve extract consistency and predictability. In this review, an attempt has been made to present *in vitro* propagation approaches used in vetiver.

Keywords: Micropropagation, vetiver, tissue culture, *in vitro* regeneration.

INTRODUCTION

Medicinal herbs are essential in the treatment and prevention of diseases. In this case, whole medicinal plants or particular portions of medicinal plants might be utilised for therapeutic purposes, which is known as Ayurveda in India, and traditional Chinese medicine in China [1]. Further, more than half of all new medications researched and licensed for sale are derived directly from modified medicinal plant products or their active ingredients [2]. India has a large plant biodiversity, including numerous medicinally precious plants. As a result of over-exploitation, this valuable resource is in decline at a worrying rate. As a result, the protection of traditional medicinal herb resources has risen to the forefront [3]. Medicinal plants have been used to flavour and preserve food for thousands of years, as well as to cure health problems and prevent illnesses such as epidemics. The medicinal abilities of these plants have been passed down the generations within and among civilized cultures [4].

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Essential oils from a variety of plants have biological characteristics such as antioxidant, anti-inflammatory, antibacterial, and genotoxic effects. As a result, in the last few years, there have been a plethora of studies on bioactive chemicals found in essential oils [5].

Vetiver grass [*Chrysopogon zizanioides* (L.) Roberty] is a perennial herbaceous plant with no visible stem [6], originating in South Asia, and widely cultivated in sub-tropical and tropical climates. Despite its economic value, it provides environmental benefits such as soil moisture retention, soil erosion minimization, and heavy metal deduction [7]. The secondary metabolites found in vetiver root extract have long been employed as a key component in the cosmetic and fragrance industries [8, 9]. Furthermore, antibacterial, herbicidal, and pesticide capabilities have been reported earlier from its extract [10 - 12].

Plant species in natural ecosystems are at risk of extinction due to natural sterility and excessive harvesting [13]. Low-cost tissue culture techniques should be regarded as a top priority in agriculture, horticulture, forestry, and floriculture of every country. Different researchers have used various ways to wisely and significantly simplify various activities involved in *in vitro* propagation in order to cut expenses while maintaining the quality of the micropropagules and plants [14]. The tissue culture technique is robust, and it has to open new avenues for biodiversity research [3]. Many novel strategies have been employed in recent years to increase the effectiveness of vetiver mass cultivation. The use of tissue culture technology for mass propagation is the most essential and crucial, since a huge number of plants may be grown in a short amount of time and at a reasonable cost [15].

MICROPROPAGATION

During the previous decades, several authors have reported micropropagation of various plant species, including many pharmaceutical herbs. Micropropagation is generally divided into multiple phases, including pre-propagation, explant initiation, explant cultures for proliferation, shoot induction, rooting, and hardening. These steps can be used in any large-scale plant multiplication situation [16]. For the regeneration of an entire plant from a cell or a callus mass, cytodifferentiation alone is insufficient; differentiation leading to organogenesis is required. This can happen *via* organogenesis (shoot bud differentiation) or somatic embryogenesis. Shoot buds (monopolar structures) develop in the former, while somatic embryos (bipolar structures) form in the latter, both leading to plant regeneration [17]. Most vetiver genotypes produce flowers but no seeds. Although a wild ecotype of vetiver grass native to southern China is fruitful, seed germination is extremely low [18]. Vetiver has conventionally been propagated

using root cuttings, which have several drawbacks, including limited viability, non-synchronized growth form, and a lower growth rate. These issues will make it difficult to transport vetiver biomass across many countries. As a result, the real need for biomass for oil extraction and biofuel production was unable to meet rising commercial needs. It is now critical to propagate and protect extinct or endangered germplasm using the most up-to-date biotechnological approach, *i.e.*, plant tissue culture [19, 20].

Table 1. Components of MS medium adapted from Dalton and Redei [38, 39].

Components	Quantity (mg/L)
Ammonium nitrate	1,650.00
Potassium nitrate	1,900.00
Potassium phosphate monobasic	170.00
Magnesium sulphate	180.69
Calcium chloride	332.20
Manganese sulphate monohydrate	16.90
Boric acid	6.20
Zinc sulphate heptahydrate	8.60
Cobalt chloride hexahydrate	0.025
Cupric sulphate pentahydrate	0.025
Molybdic acid	0.213
Potassium iodide	0.83
Ferrous sulphate heptahydrate	27.80
Nicotinic Acid	0.5
Pyridoxine HCl	0.50
Glycine	2.0
Myo-Inositol	100.0
Thiamine HCl	0.1
Disodium EDTA dihydrate	37.26
Carbon source-sucrose	30000.0

Vetiver Explant Source

After a year in the nursery, a sharp sterile stainless steel scalpel has been used to slice the stalks with nodes carrying axillary buds [21]. A methodology for regeneration systems employing leaf, node, and root explants *via* organogenesis and somatic embryogenesis has been established [13]. Despite the reality that a

CHAPTER 16

Micropropagation of *Actinidia deliciosa* (A. Chev.) C.F.Liang & A.R.Ferguson

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Abstract: *Actinidia deliciosa*, commonly known as Kiwifruit (Chinese gooseberry), belongs to the family Actinidiaceae. The edible and fleshy fruit has gained popularity over the past few decades owing to its high nutritive value, and medicinal and potential curative properties. The fruit is rich in vitamin C, folate, vitamin E, dietary fibers, antioxidants, enzymes, phytonutrients, *etc.* The presence of actinide in Kiwis helps in regulating gastric abnormalities, hypertension, cardiovascular inflammation, hemostatic disorder, and abnormal glucose metabolism, and prevents cancer. Consequently, the fruit holds a considerable market value that has led to the establishment of industrial organizations comprising growers and distributors. For the purpose of fulfilling the constant market demands, it is crucial to maintain quality standards, timely production, and an abundance of planting material. This chapter discusses the various *in vitro* propagation methods, including diverse and detailed approaches for both the direct and indirect organogenesis for large-scale production of good-quality kiwi plants, along with *ex vitro* hardening and acclimatization processes. It is apparent that the plant tissue culture techniques can be suitably applied for the mass production of kiwi fruit, while other *in vitro* manipulations and further biological research are needed to improve the field performance and post-harvest life of the fruit and its plant.

Keywords: *Actinidia deliciosa*, kiwi, axillary bud culture, indirect organogenesis, anther culture, embryogenesis, hardening.

INTRODUCTION

Actinidia deliciosa (A.Chev.) C.F.Liang & A.R.Ferguson, commonly known as Kiwi, is a large deciduous vine well known for its edible fruit [1]. Kiwi is a woody unisexual grape that grows up to 10 m in height. A kiwi plant bears either white to cream-colored male or female flowers that give way to oval fruits. To ensure efficient cross-pollination between males and females, they are planted together in a ratio of 8:1 (female: male) in a vineyard [2]. The end of the winter

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season is considered best for budding; budding lasts for almost 15 days. If the conditions are favorable for male plants, they bloom throughout the season with female flowering plants. Although kiwi is a native of Taiwan and China, it is cultivated widely across the globe. Though the plant prefers warm and temperate climates, it can tolerate a wide range of conditions. The leading producers of kiwi around the world are California, New Zealand, and Italy, while countries like Israel, France, Spain, *etc*., harvest kiwi as well.

Fig. (1). (**a**). Kiwi fruit; (**b**). seeds and inner flesh of fruit.

Kiwi fruit is ovate, 55–70 mm long, with green flesh and has light brown skin (Figs. **1a** & **1b**). The ripe fruit is very juicy and has the characteristic sour taste of a mixture of pineapple and strawberry. Kiwis are densely rich in nutrients and are remarkably high in vitamin C as compared to other citrus fruits. Also, a significant amount of other nutrients, like vitamin E, dietary fibers, potassium, and folate, are rich in vitamin C, bioactive compounds, and various nutrients. Apart from these, a variety of phytonutrients, enzymes, and antioxidants are found in Kiwi, which have an essential role in the metabolic pathways in humans. Due to the richness of actinide, it plays an active role in digestive health in humans [3]. *Actinidia* has a natural proteolytic enzyme that aids in gastric digestion by breaking down proteins and triggering the mobilization of phytochemicals [4, 5]. Kiwifruit possesses anticancer properties and cardioprotective properties. Kiwi is also known to improve hypertension, inflammation, and hemostatic disorders [6]. These nutritive benefits of the kiwi fruit have resulted in high consumer demand calling for a sustained supply throughout the year [7].

NEED FOR TISSUE CULTURE OF KIWI

Within a few decades, the micropropagation of fruit crops has become a commercial reality from just being a theoretical possibility. Initially, the techniques of fruit micropropagation were applied to strawberries [8] and subsequently to other fruit crops, including raspberry, blackberry, cherry, plum, peach, apple, and pear [9]. The focussed work on kiwi was initiated by Young [10] who emphasized the growing economic importance of Kiwi and the need for mass expansion of kiwifruit production [11].

The genus *Actinidia* is itself very diverse [12] and the commercial production is based on the cultivar (cv.) 'Hayward'. Although improvements have been made, the cultivar is not free of inadequacy and exhibits problems of disease occurrence at a very high rate, frost damage, or pest infestation, which occurs due to slender genetic variability and the dioecious nature of the fruit [13]. Through micropropagation of cv. Hayward and various *in vitro* techniques the quality and stress tolerance capability of (cv.) can result in the production of a much more efficient commercial variety.

With these observations, it has been suggested that micropropagation of kiwi holds significance for producing this commercially significant species at a very large scale. This chapter reviews the micropropagation techniques through which the existing problems of propagation can be resolved and an enormous amount of planting material can be prepared for commercial usage.

METHODS OF MICROPROPAGATION

Plant tissue culture refers to the technique of growing cells, tissues, or organs of plants in *in vitro* aseptic conditions to regenerate the whole plant in a nutritionally controlled environment [14]. The technique is useful where the conventional methods of propagation are slow, difficult, and laborious. In general, explants like nodes, shoot tips, whole buds, and shoot meristems are commonly used for this purpose [2, 15, 16]. The first-ever *in vitro* propagation procedure for Kiwi was suggested by Harada in the year 1975 [17] and later improvised by Monette [18], Wang *et al*. [19], Wessels *et al*. [20], and Standardi [21]. Harada [17] in his work found that the morphogenic response of the explants varied according to the type of, the combination of, and the concentration of plant growth regulators (PGRs) that were supplemented to the nutrient medium. He obtained four kinds of organogenetic responses, which were bud formation, root induction, and differentiation of globular embryoids. For obtaining shoot buds, the most suitable medium was found to be supplemented with 1mg/L of Zeatin, and the male explants showed relatively higher organogenetic capacity than the female explants. Later, the newly formed shoots (1cm long) were soaked in 1mg/L IBA

CHAPTER 17

An Update on Biological, Pharmaceutical, and Biotechnological Investigations in *Pterocarpus marsupium* Roxb.

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Abstract: *Pterocarpus marsupium* Roxb. is one of the important plants of the Fabaceae family and is present in different regions of the world. It is greatly valued for its medicinal properties and has often been used for medical purposes. It was observed that *P. marsupium* contains numerous phytochemical components, such as glycosides, proteins, cardiac glycosides, terpenoids, alkaloids, carbohydrates, and flavonoids. Due to overexploitation, the natural population of *P. marsupium* is declining steadily, because of which it is required to be cultivated on a larger scale. The conventional propagation methods of *P. marsupium* are time-consuming processes, and the plant is not easy to propagate through seeds because of its low germination percentage. Hence, to overcome the problem related to conventional propagation and to reduce the destruction of plants in wild habitats, tissue culture functions as an important tool to conserve the plant. The tissue culture practice is extremely useful to meet the rising demands of the people because it gives a significant number of elite genotype progenies within a limited time and without seasonal dependence.

Keywords: Anti-diabatic, Genetic fidelity, Hepatoprotective, *Pterocarpus marsupium*, Pterostilbene, Stilebene.

INTRODUCTION

Pterocarpus marsupium, commonly called Malabar Kino or Indian Kino, has its place in the Fabaceae family and is the most important leguminous tree characterized by a high yield of valued timber with fast growth rate and disease resistance. It is native to southern and eastern Asia, particularly Nepal, Bangladesh, Sri Lanka, India, and Taiwan. The wood of *Pterocarpus* has often been compared with teak but has gained less attention for its poor cultivation. Due

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to the neglected cultivation and fast decline in its wild due to indiscriminate cutting, the tree has been placed in near threatened category of the red data book of the International Union for Conservation of Nature and Natural Resources (IUCN) [1]. *P. marsupium* is greatly valued for its medicinal properties and has often been used for medical purposes implicitly. One of its compounds, namely gum kino, is extracted from the tree and has been broadly used against diseases like dysentery, fever, diarrhea, and toothache. Phenolic compounds like pterostilbene and marsupium obtained from the heartwood of the tree have been reported to have anti-hyperglycemic activities [2]. It was found that the aqueous extract of the bark of the stem significantly reduces the level of glucose in alloxan-influenced diabetic rats [3]. Similarly, 'Vjayasar', an herbal product taken out from the tree bark, has given positive outcomes while treating diabetes. Flavonoid constituents of *P. marsupiums,* such as marsupsin, pterosupin, and liquiritigenin are helpful in reducing cholesterol and hence act as anti-hyperlipidemic [4]. Some tribal groups living in the Joldhal forest area of Karnataka use stem bark for the treatment of stomachache, jaundice, fever, and diabetes. The flavonoid content of the plant consists of antioxidant characteristics, which is useful in treating liver damage [5]. The usual regeneration of the plant takes place with the help of seeds, but it has been reported that the percentage of seed germination is very low, almost 30% [6], and thus, its propagation through seeds has been found to be unsuccessful because of its rigid fruit coat, less germination potential and minimum viability [7]. *P. marsupium* has been entered into the category of declining species because of its exploitation at an alarming rate coupled with less propagation and low germination rate [8]. Therefore, efforts have been made by scientists to develop an easy protocol for the effective *in vitro* culture of *P. marsupium*.

PHYTOCHEMICAL CONSTITUENTS PRESENT IN *PTEROCARPUS MARSUPIUM*

While reviewing the literature, it was observed that *P. marsupium* contains numerous phytochemical components such as glycosides, proteins, cardiac glycosides, terpenoids, alkaloids, carbohydrates, and flavonoids. Similarly, studying the antidiabetic, antihyperlipidemic, and antioxidant activities of wood and bark ethanol extract of *P. marsupium* confirmed the presence of alkaloids, glycosides, flavonoids, saponins, steroids, and coumarins [9]. The heartwood and plant root contain phytochemical like pterosupin, liquiritigenin, pentosan, garbanzol, 5-de-oxykaempferol, p-hydroxybenzaldehyde, isoliquiritigenin, eryth-rodirol-3-monoacetate, propterol B, and marsupinol [10]. Moreover, the wood of the plant consists of a dyeing material of yellow colour, a semi-drying fixed oil, and essential oil. Similarly, a gum-Kino can be extracted through a cut made up to the cambium of a bark [11]. Furthermore, in terms of health benefits, among all

the compounds extracted from the plant, phenolic and flavonoid compounds were found to be the most significant [12]. The structure and quantity of phytochemicals found in medicinal plants can differ from one place to another, growing circumstances, and age. Hence, the study of the phytochemical profile of various parts of the plants is vital in order to assure the effectiveness, protection, legitimacy, and value of products. The quick qualitative and quantitative assessment of identified compounds spotting anonymous compounds in small quantities extract and formulation have been useful and prevalent and magnificently finished by liquid chromatography-mass spectrometry (LC-MS). However, no LC–MS report exists for recognising the phytochemical presence in *P. marsupium,* excluding pterostilbene metabolite in the urine of the mouse.

Compounds reported in *P. marsupium* include lupeol, vijyayosin, hypanthorin [13], garbanzol [14], 2,6-dihydroxyphenylglucopyranoside, ptersupol, marsuposide [15], isoliquiritigenin [16], pterosillbene [17], sesquiterpene [18], propterol [19], pterosupin, epicatechin, liquirtigennin, pterosupin [4], naringenin [20] and carpusin [21].

PHARMACOLOGICAL PROPERTIES OF *PTEROCARPUS MARSUPIUM*

The modern tool of biotechnology is helpful in detecting, extracting, and enhancing the medicinal compounds of plants. Conventionally, compounds from barks serve as prominent sources of medicines and raw ingredients. Several compounds obtained from the bark display inclusive medicinal properties and help in diagnosing the several disorders associated with human well-being [23]. Similarly, screening of phytochemical compounds and learning about antioxidant, antidiabetic, anti-inflammatory, antimicrobial, and analgesic activities of extracts from stem wood of *P. marsupium* display vital consequences in treating several human health disorders [22].

Antibacterial Activity

Assessment of the antimicrobial activity of *P. marsupium* took place in an *in vitro* condition against the pathogenic bacteria *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Staphylococcus aureus*. The small inhibitory concentration of 0.04 mg and 0.08 mg of aqueous extract of *P. marsupium* was effective against bacterial growth, and *Staphylococcus aureus* was found to be more susceptible against this aqueous extract [10]. In another experiment, the antimicrobial potential of methanolic extract of stem bark of *P. marsupium* was estimated against *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhi*, *Pseudomonas aeruginosa,* and *Micrococcus* sp. Here, among the three different regions of bark, *i.e*., apical bark, middle, and mature bark, the apical one was observed to be more responsive and

CHAPTER 18

Micropropagation in *Balanites aegyptiaca* (L.) Del.

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Abstract: *Balanites aegyptiaca* belongs to the family Zygophyllaceae, present in tropical countries of the world. This plant is well known for its medicinal properties. *B. aegyptiaca* contains numerous phytochemical components such as glycosides, proteins, steroids, terpenoids, alkaloids, and flavonoids. Due to overexploitation, the natural population of plants is declining in the wild. Also, conventional propagation of the plants is not sufficient in terms of the production and the number of the plants. Therefore, to reduce the problem associated with traditional propagation and production of plants on a larger scale, *in vitro* propagation is the most suitable approach. During *in vitro* propagation, a sufficient number of elite genotype progenies within a limited time period and without seasonal dependence are produced.

Keywords: Diosgenin, micropropagation, somatic embryogenesis, synthetic seed.

INTRODUCTION

Balanites aegyptiaca is an evergreen xerophytic tree mostly found in tropical countries and Syria, Sudan, Egypt, West Asia, and many West African countries such as Nigeria, Senegal, Myanmar, India and Arabia. The tree is well adapted to various agroclimatic areas, particularly to the areas specified by arid and semi-arid climatic features [1]. Various studies have reported that the plant possesses a range of medicinal properties such as anti-fungal, anti-leishmanial, anti-bacterial, anti-molluscidal and anti-cancer properties [2, 3]. Traditionally, the plant parts of the tree, such as roots, bark, shoots, leaves, kernel oil, fruits and seeds, have been used to treat various kinds of diseases such as sleeping sickness, guinea worm disease, whooping cough, skin disorders, *etc.* The medicinal potential of the tree perhaps lies in the extraction of some important secondary metabolites like Diosgenin, which serves as a partial material for the synthesis of sex hormones, oral contraceptives, and other various steroids [4]. Moreover, the tree is drought tolerant, and the kernel of the plant has been successfully used for biodiesel production [5].

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An unscientific approach coupled with limited cultivation of the species has significantly depleted its wild stock. Conventionally, *Balanites* has been propagated by vegetative methods involving seeds and root suckers, however, these methods have turned insufficient in terms of production number and higher frequency of seed germination, besides a low survival rate in the plant cuttings during the plantation process. The application of biotechnology on trees has opened up alternatives for the propagation of the plant in large numbers as well as conserving the germplasm of various medicinal plants. In general, the *in vitro* regeneration of a tree species is a difficult task; however, some success has been achieved [6]. Seeds are believed to be the units of recombination, thus, plants regenerate from them will differ genotypically from the mother plant. Therefore, in order to establish clonal lines of *B. aegyptiaca* as well as maintain the stock of the species for conservation and wood purposes, the *in vitro* approach is a best practice to opt for.

MICROPROPAGATION OF *BALANITES AEGYPTIACA*

Naturally, *Balanites aegyptiaca* is regenerated by seeds, root suckers, and coppicing; however propagules' growth is slow. Because of the labor-intensive nature of traditional propagation methods, as well as the necessity for broad regions in ex-situ conservation, out-breeding, long juvenile period and periodic dependence, *in vitro* technologies are required. However, there are a few ways for generating *in vitro* plants in *B. aegyptiaca* utilising various explants, namely nodal segments, axillary buds, and shoot tips [7 - 10].

Direct Organogenesis

Multiple shoot formation is a very important technique of micropropagation and is used for the production of a large number of plants that can be used in the treatment of some diseases because some diseases are cured by the use of direct plant parts, production of the secondary metabolites which retain in plant parts or to enhance the particular product of that plant. In *B. aegyptiaca,* shoot multiplication was done through direct organogenesis by using different types of explants by various researchers. For example, Hussein *et al*. [11] used Mammalian Sex Hormones (estrogen, progesterone, human chorionic gonadotropin (HCG), testosterone, and anabolic steroids) on the nodal segment to enhance the regeneration of *B. aegyptiaca*. This experiment showed positive effects on shoots number per explant, shoot length, number of leaves per shoot, and leaf area. Mostly, *in vitro* regenerated explants like apical buds, nodal segments, young thorns, cotyledon explants, and seedling derived roots were used by the researchers for direct organogenesis. These plantlets were regenerated through seed germination on Murashige and Skoog (MS) [12] medium [8, 13 -

15]. Mostafa and Alhamd [15] used (gibberellic acid) GA_3 and (indole-3-acetic acid) IAA to improve the growth and chemical constituents of *B. aegyptiaca* and found that 50 ppm of GA_3 and 2000 ppm of IAA are the best for increasing the growth and chemical constituents of the plant. Likewise, Mekawy [16] used some additives (adenine sulphate, diphenyl urea and phlorglucocinol) to investigate the effect on micropropagation of *B. aegyptiaca.* The most effective treatment was MS + (kinetin) Kn (1 mg/l) + (1-Naphthalene acetic acid) NAA (0.2 mg/l) + phlorglucocinol (8 mg/l) to enhance the growth of nodal and shoot tip explants.

Varshney and Anis [17] used MS with various cytokinins [(benzyl adenine) BA, Kn, (2-Isopentyl Adenine) 2-iP] at various concentrations (1.0, 2.5, 5.0, 10.0, 12.5, 15.0 μM) either singly or with different concentrations (0.5, 1.0, 2.0, 2.5 μM) of auxins [NAA, IAA, Indole-3-butyric acid (IBA)] for root culture to achieve shoot organogenesis. In cytokinins alone, BA (5.0μM) was found to be the best, and a combination of MS + BA (5 μM) + NAA (1.0 μM) gave the highest frequency (68%) of shoot regeneration with maximum shoot number (7.20 ± 0.15) per explant. Different steps of micropropagation through direct organogenesis are shown in Fig. (**1**).

Indirect Organogenesis

Callus-mediated organogenesis has been done because it has several advantages like callus can be used to investigate developmental biology [18] and somaclonal variation [19], and also for cell suspension culture to produce SMs [20]. Indirect organogenesis has been a general method for the micropropagation of a plant. Ebad *et al*. [21] performed the callus formation to investigate the effect of some nanoparticles (AgNPs, ZnPs, CuNPs) at some different concentrations (1, 5, 10, 20 mg/l) on morphology, growth and some active SMs in callus culture of *B. aegyptiaca* (L.). Gour *et al*. [8] also used callus mediated organogenesis by inoculating different explants (apical, buds, young thorns and cotyledon pieces) collected from *in vitro* raised seedlings, on MS + 2,4-Dichlorophenoxyacetic acid (2,4-D) (2.23 μM).

A complete description of micropropagation of *B. aegyptiaca* is detailed in Tables **1** and **2**.

Explant selection

Explant selection is a very crucial step of *in vitro* propagation because the physiological condition, choice, size, position or orientation of the stock plant affects the result. In *B. aegytiaca,* explants are used either from field-grown plants or *in vitro* regenerated plantlets. Khamis *et al*. [13] used *in vitro* regenerated nodal segments and cotyledons explants for direct organogenesis, while Anis *et al*. [10]

CHAPTER 19

In vitro Propagation of *Ruta graveolens* L.

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Abstract: *Ruta graveolens* L., a multipurpose perennial herb, belongs to the family Rutaceae. It is a native of the Mediterranean region and is commonly known as Garden Rue or Herb of Grace. From time immemorial, Rue has been known for its rich aromatic and medicinal properties. More than 120 compounds of different classes of natural products, such as acridone alkaloids, coumarins, essential oils, flavonoids and furanoquinolines, have been isolated from *R. graveolens*. Having a vast range of secondary metabolites, this plant has been used worldwide for several therapeutic usages. The essential oil obtained from the distillation of the entire plant has several therapeutic values. The entire plant is used as an abortifacient, anthelmintic, antispasmodic, carminative, emmenagogue, expectorant, haemostatic, ophthalmic, and rubefacient. Besides pharmaceutical applications, this plant is used in cosmetics and food items. Ripened fruits are used as condiments and leaves are used to make pickles. Several effective protocols for micropropagation have been developed by several researchers. Due to its vast usage, the plant is disappearing in the wild. Conventional propagation methods do not meet the market demands. Hence, there is an urgent need to shift to *in vitro* methods for quick and genetically elite plant production. In this chapter, detailed protocols for *in vitro* propagation methods are discussed.

Keywords: *In vitro* propagation, micropropagation, secondary metabolites.

INTRODUCTION

Ruta graveolens is an herbaceous ornamental perennial shrub with blue-green foliage and yellow flowers and is commonly known as Garden Rue or Herb or Grace. It is a strongly scented medicinal and aromatic plant commonly growing in the Mediterranean region [1]. Though it is a native of the Mediterranean region, now it is grown worldwide as an ornamental plant as it is tolerant to dry soils and hot climatic conditions [2]. It was famous in ancient Roman times, the Middle Ages and the Renaissance. All parts of the plant are medicinally useful. A pale

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yellow or greenish volatile oil is produced through steam distillation of the fresh material. Seeds contain high amounts of volatile oil compared to other parts of the plant [3]. Rue oil has a strong odor and a bitter pungent taste. Often, it is adulterated with turpentine oil and petroleum [4, 5]. The whole plant and its essential oil are widely used for various therapeutic usages. The volatile Rue oil possesses antibacterial activity against *Micrococcus pyogenes* var. *aureus* and *Escherichia coli*. The essential oil is also used in the preparation of perfumes and soaps as it is rich in methyl-nonyl-acetaldehyde. The essential oil contains several biologically active compounds, such as bergaptene, butanone, nonanone, nonyl acetate, psoralen, undecanone, and xanthotoxin [1]. The entire plant is used as an effective abortifacient, anthelmintic, antispasmodic, carminative, emmenagogue, expectorant, haemostatic, ophthalmic, and rubefacient [6 - 8]. Rue's active ingredients have antifungal properties, which could be beneficial to agriculture and medicine [9].

The Rue plant is especially used to cure several skin diseases like psoriasis and vitiligo. Furanocoumarins, especially bergapten, have been tested for psoriasis and vitiligo [10, 11]. The whole plant is applied externally as a poultice against rheumatic pain [12]. The plant is also being studied in the context of neural disorders like multiple sclerosis [13]. The flavonoid content of the herb has been reported to possess antibacterial [14] and cytotoxic activity [15]. Acridone alkaloids obtained from this plant showed significant antiviral [16] and antiplasmodial activity [17]. Rue extracts have been proposed as topical pharmaceutical fungicides [18, 19]. The flavonoid rutin present in the Rue plant possesses nitric oxide scavenging activity [20], thereby suggesting the alleged anti-inflammatory property of the plant.

More than 120 compounds of different classes of natural products, such as acridone alkaloids, coumarins, essential oils, flavonoids, and furanoquinolines, have been isolated from *R. graveolens*. Petit-Paly *et al*. [21] published a detailed review on chemical constituents present in *R. graveolens*. Several volatile chemical constituents have been isolated by Kubeczka [22]. A large number of coumarins and alkaloids have been isolated by several researchers [23 - 30].

Poutaraud *et al*. [31] did experiments on the best harvest time of the *R. graveolens* crop to get a higher concentration of furanocoumarins in the biomass of the plants. They identified that when the plant has a high leaf and fruit biomass, it contains high amounts of furanocoumarins. The relative proportion of ligneous stems limits the content of furanocoumarins. They suggest that a minimum of three cuts per year is needed to get high furanocoumarin content in the biomass.

IN VITRO PROPAGATION

Need to go for *In Vitro* Studies

Pharmaceutical companies collect the plant material in large quantities from the natural habitat. Hence, the existence of this plant in the wild will be in critical condition. Naturally Rue plant is propagated by seeds and vegetative methods. Multiplication through seeds and vegetative methods is insufficient to meet the market demands. Poor germination percentage and low viability limits the production of nursery plants for cultivation. Moreover, no significant endeavor is being made for the cultivation and replenishment of the wild stock. Hence, the conservation of wild genetic stock is badly needed for future needs. Production of planting material for commercial mass propagation is a big task. Because the quality of plant material is not uniform from place to place. Therefore, the production of elite nursery plants for commercial is highly essential. Micropropagation is the only means to meet the market demands. And *in vitro* production of secondary metabolites is another cost-effective alternative to meet the growing demand.

In vitro Propagation Studies

Keeping in view the increasing demand for *R. graveolens*, several researchers developed protocols for the quick multiplication of the Rue plant. Castro *et al*. [32] attempted to propagate *R. graveolens* from nodal segments cultured on half strength Murashige and Skoog (MS) medium supplemented with BAP (0.1 mg/L). Mishra *et al*. [33] cultured nodal explants excised from the matured plants on MS medium supplemented with different concentrations of BA and found that maximum number of shoots (14-18) was at 3 mg/L concentration. The excised shoots were cultured on half-strength MS medium supplemented with different concentrations of Indole-3-Butyric Acid (IBA) and found that effective rooting was at 3 mg/L.

Faisal [34] developed a rapid clonal propagation method by culturing nodal segments on MS medium supplemented with different concentrations of Benzyl adenine (BA) and kinetin (Kn) (0.5 to 20 μM) either alone or in combination with auxins [Indole-3-Acetic-Acid (IAA) and Naphthalene Acetic Acid (NAA) from 0.5 to 5 μM]. The two cytokinins when tested alone promoted axillary bud sprouting and the best response was observed on the medium supplemented with 10 μM BA (83.8% of response and 22.2 shoots per explant). The addition of IAA or NAA increased the percentage of response and number of shoots per explant. The data revealed that NAA was effective than IAA. The highest percentage of response (98.5) and the highest number of shoots per explant (40.2) were observed on the medium supplemented with 10 μM BA and 2.5 μM NAA. The

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