PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA
MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH
MOHAMED BOUDIAF UNIVERSITY – M'SILA

FACULTY OF SCIENCE

DEPARTMENT OF NATURAL AND LIFE SCIENCES

N° :……………………………………………..

FIELD: Natural and Life Sciences
MAJOR: Biotechnology
OPTION : Plant Biotechniology

Thesis
Submitted in candidacy for the degree of Academic Master's degreeBy:

ALI ZEGHLACHE Cheyma & BOUTI Ferial

Title

In *vitro* culture of medicinal plants

In front of the following panel of examiners:

P.Ghadebane Mouloud	Univ. of **M'sila**	**President**
P. ADOUI Nabila	Univ. of **M'sila**	**Supervisor**
Dr. HADJI Maroua	Univ. of **M'sila**	**Co-Supervisor**
Dr. Belkassem Abdelouahab	Univ. of **M'sila**	Rapporteur.

Academic year: 2024 /2025

Acknowledgments

First and foremost, we express our sincere gratitude to Allah Almighty for granting us the strength, patience, and perseverance to complete this work. Praise and thanks be to Allah.

We would like to extend our heartfelt thanks and appreciation to our supervisor, Dr. ***Nabila ADOUI****, for her invaluable guidance, support, and insightful direction throughout this research. Our sincere thanks also go to our co-supervisor, Dr.* ***Maroua HADJI,*** *whose support and constructive input had a significant impact on the quality of this work.*

We are especially grateful to Professor ***Hamdi BENDIF****, whose continuous encouragement and guidance have greatly enriched our academic journey. His support has been truly invaluable.*

We also express our appreciation to our rapporteurs, Pr. ***Benkacem*** *and* ***Pr. Ghadbane****, for their time, efforts, and valuable evaluations of our work.*

We further thank all our friends and colleagues in the 2024/2025 Master's program in Plant Biotechnology for their collaboration, motivation, and companionship throughout this period.

Finally, we extend our sincere thanks to everyone who contributed—directly or indirectly—to the completion of this work.

Dedication

We dedicate this humble work to our dear parents,
Who have always been our true support and the first source of encouragement at all stages of our lives.

And to our dear uncles, aunts, and relatives,
Who have showered us with their love, prayers, and constant support.
We also dedicate this work to our dear friends who have shared this journey with us:
Ferial's family: To my beloved family, especially my dear maternal uncles, thank you for your constant presence and sincere love. You are my support and my pride.

My sisters / brothers :Hadil , Mohamed , My maternal cousins.

Feryal's friends: Sara, Fatna, Moussa, Ramzi, Said, Hichem, Slimane,Ali,Nouha

Chaima's family : dear husband ali djeroui and all his family

My sisters / brothers : asma, siham, louiza,abd elkader, abd elrahmen, ismail

Our friends: Besma ,Meriam, Hiba , Sameh, selma , Habiba, Asma.

To all of you, our heartfelt thanks and gratitude,

And we pray that Allah blesses your efforts and grants you continued success, prosperity, and everlasting happiness .

Abstract:

In *Vitro* culture of medicinal plants has emerged as a pivotal biotechnological strategy for the sustainable production, conservation, and exploitation of plant-based therapeutic resources. This technique enables the sterile and controlled cultivation of plant cells, tissues, and organs, facilitating the mass propagation of genetically stable and disease-free plants. It serves as a reliable platform for producing high-value secondary metabolites, which are extensively used in the pharmaceutical, nutraceutical, and cosmetic industries. In addition to its role in commercial-scale production, in *vitro* culture contributes significantly to phytoremediation by enhancing the pollutant-absorbing capabilities of selected plant species. Overall, the integration of in *vitro* culture techniques into medicinal plant biotechnology provides a promising solution for meeting the growing global demand for natural health products while supporting environmental sustainability and resource conservation.

Keywords: In *vitro* culture, medicinal plants, biotechnological strategy, biodiversity**,** plant tissue culture

Résumé :

La culture in *vitro* des plantes médicinales s'est imposée comme une stratégie biotechnologique clé pour la production durable, la conservation et l'exploitation des ressources thérapeutiques d'origine végétale. Cette technique permet la culture de cellules, tissus et organes végétaux dans des conditions stériles et contrôlées, facilitant la multiplication massive de plantes génétiquement stables et exemptes de maladies. Elle constitue une plateforme fiable pour la production de métabolites secondaires de grande valeur, largement utilisés dans les industries pharmaceutique, nutraceutique et cosmétique. Par ailleurs, elle joue un rôle crucial dans la conservation et la multiplication des plantes médicinales menacées, assurant ainsi la préservation de la biodiversité et réduisant la pression sur les populations sauvages. L'intégration des techniques de culture in *vitro* dans la biotechnologie des plantes médicinales offre ainsi une solution prometteuse pour répondre à la demande mondiale croissante en produits de santé naturels, tout en soutenant la durabilité environnementale et la préservation des ressources.

Mots-clés : Culture in *vitro*, plantes médicinales, stratégies biotechnologiques, biodiversité, culture de tissus végétaux

ملخص

تُعد زراعة النباتات الطبية في المختبر (*In vitro*) من أهم الاستراتيجيات البيوتكنولوجية التي ساهمت في الإنتاج المستدام، والحفاظ على الموارد النباتية العلاجية، واستغلالها بشكل فعال. تتيح هذه التقنية زراعة الخلايا والأنسجة والأعضاء النباتية في ظروف معقمة ومتحكم بها، مما يسهل الإنتاج السريع للنباتات السليمة والثابتة وراثيًا والخالية من الأمراض. كما توفر منصة موثوقة لإنتاج المركبات الثانوية ذات القيمة العالية ، والتي تُستخدم على نطاق واسع في الصناعات الصيدلانية والتجميلية والغذائية. إلى جانب دورها في الإنتاج التجاري. كما تلعب هذه التقنية دورًا محوريًا في حفظ وإكثار النباتات الطبية النادرة والمهددة بالانقراض، مما يساهم في الحفاظ على التنوع البيولوجي وتقليل الضغط على الموارد الطبيعية البرية. وبذلك تمثل زراعة النباتات الطبية في المختبر حلاً واعدًا لتلبية الطلب العالمي المتزايد على المنتجات الطبيعية، مع دعم الاستدامة البيئية والحفاظ على الموارد الحيوية.

الكلمات المفتاحية الاستراتيجيات البيوتكنولوجية , النباتات الطبية ,التنوع البيولوجي, زراعة في المختبر, زراعة الأنسجة النباتية

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

N: Nitrogen
P: Phosphorus
K: Potassium
S: Sulfur
Mg: Magnesium
Ca: Calcium
B: Boron
Mn: Manganese
Zn: Zinc
Cu: Copper
Ni: Nickel
Co: Cobalt
Mo: Molybdenum
Al: Aluminum
I: Iodine
Fe: Iron

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Introduction

Since ancient times, plants have played a fundamental role in traditional medicine, serving as the basis for numerous treatments for various ailments. The bioactive compounds found in medicinal plants such as alkaloids, flavonoids, terpenoids, and other secondary metabolites are widely used for their therapeutic potential **(Pan et al. 2013; Wink, 2015)**.

Today, despite advances in pharmaceuticals, medicinal plants remain a valuable source for the development of new drugs and treatments. Their use in modern medicine has been supported by numerous scientific studies that have demonstrated their potential in treating a wide range of diseases, from infections to chronic disorders **(Rates, 2001; WHO)**.

In response to the growing human demand for medications, plant tissue culture is widely used for the micropropagation of medicinal plants, enabling the production of sufficient quantities of active compounds and secondary metabolites. This technology offers the advantage of providing natural products year-round, independent of seasonal constraints, while allowing optimal control over growth conditions. Furthermore, it enables the production of these compounds in a relatively short time and in a limited space **(Ramachandra and Ravishankar, 2002; Murthy and Paek, 2014)**.

Therefore, our study aims to explore and summarize the techniques used for the *in vitro* culture of plants such as micropropagation, tissue culture, and somatic embryogenesis. The thesis will discuss the advantages and limitations of these techniques, as well as their potential applications for medicinal plants.

Based on this context, our work is presented in three core chapters:

- The first chapter covers the general aspects of in vitro culture;
- The second focuses on medicinal plants, and their secondary metabolites;
- The third explores the applications of in vitro culture in medicinal plants.

Finally, the conclusion summarizes the main research findings and offers perspectives for future research.

CHAPTER ONE

IN VITRO CULTURE

I. I.1. General aspects of In vitro culture

I.1Definition

In vitro culture**,** or plant tissue culture, is a technology of growing plants under sterile conditions in a controlled environment. The latter is provided by using a laboratory-prepared medium with known concentrations of nutrients, vitamins and growth regulators and growth chambers with controlled light, temperature and humidity. While achieving better control over plant development and health status, in vitro culture requires less space compared to field collections and hence is often used to safely duplicate accessions of clonally propagated crops that cannot be conserved as seeds **(CGIAR, 2013)**

In vitro culture techniques owe their development to the fundamental principle of plant propagation: totipotency**,** which allows undifferentiated cells to develop into a whole plant. This concept, introduced by Haberlandt in 1902, forms the very foundation of in vitro culture **(Auge et *al*., 1989).** This technique includes two modes of multiplication (conform and non-conform). Currently, only axillary budding (growth from meristems located at the leaf axils) and adventitious budding **(**induction of shoot meristems on organs that do not naturally possess them) are developed for forest species, particularly *Pinus radiata*. Experiments with other methods are still in their early stages. **(Iliev, 2017)**. Several stages are involved in in vitro micropropagation**.** Some resemble traditional multiplication techniques (pre-treatment of the mother plant, rhizogenesis, acclimatization), while others are specific to in vitro culture (selection of the most responsive explant, sterilization of the plant organ, choice of propagation technique) **(George, 2008)**

I.1.1. History

In vitro plant culture has evolved significantly since its beginnings in the early 20th century. Gottlieb Haberlandt laid the theoretical foundations in 1902 by introducing the concept of totipotency—the ability of a plant cell to differentiate into a whole plant. His pioneering work set the stage for in vitro plant culture **(Haberlandt, 1902)**.

During the 1930s and 1940s, researchers such as White (1934) and Gautheret (1939) successfully cultivated plant tissues on artificial nutrient media. The discovery of plant growth regulators, particularly auxins and cytokinins, by Skoog and Miller (1957) revolutionized in vitro culture by enabling precise control over tissue growth and differentiation **(Skoog and Miller 1957).**

The 1950s and 1960s saw the development of advanced techniques such as callus culture, organogenesis, and somatic embryogenesis, facilitating the complete regeneration of plants from tissue fragments. By the 1970s, micropropagation had expanded rapidly, making it possible to produce genetically identical plants on a large scalean essential advancement for agriculture and forestry **(George et al., 2008)**.

More recently, *in vitro* culture has been used to study plant adaptation to climate change. For example, in 2023, the New Caledonian Agronomic Institute (IAC) utilized this technique to analyze drought tolerance in Oxanthera spp., an endemic citrus species, aiming to understand its adaptation mechanisms (iac.nc).

In 2022, a detailed study highlighted the advantages and limitations of *in vitro* culture for date palms (*Phoenix dactylifera L.*), emphasizing its importance in rapidly meeting plant production needs, surpassing traditional propagation methods **(Benderradji, 2022)**.

Today, *in vitro* culture is widely used for plant propagation, genetic engineering, conservation of endangered species, and production of disease-free plants. Advances in biotechnology, including genetic transformation techniques and genome editing via CRISPR, continue to expand the applications of in vitro culture, making it an indispensable tool in modern plant science and agriculture.

I.1.2. Totipotency

The theory of cellular totipotency was first proposed by **Haberland** in 1902 and later revisited by **Steward** in 1967. This theory is based on the principle that all the genetic information required for the embryogenesis process is contained within the nucleus of each living cell **(Norrel, 1973)**. Dedifferentiated tissues or cells have the ability to develop in various directions, demonstrating the pluripotency of plant cells. This property is also found in germ cells, such as pollen grains and ovules. Since

1966, this approach has been successfully used, particularly in obtaining haploids, whose chromosomes can be doubled through the action of cholinase. This characteristic also serves as the basis for the successful isolation and culture of protoplasts **(Djennane and Klifatti, 1996).**

In theory, each protoplast has the ability to regenerate a plant identical to the female parent. Even highly differentiated plant cells can generally be restored to this primitive state. A differentiating cell can thus resume a division process and organize itself into a new structure depending on its environment and the specific conditions to which it is subjected **(Djennane and Klifatti, 1996).**

Cellular totipotency can be experimentally reactivated through a process of dedifferentiation. This phenomenon can be triggered by trauma, by the action or inhibition of phytohormones, or by the destruction of existing organic correlations **(Demarly and Sibi, 1996).** Totipotency is therefore an intrinsic property of every viable plant cell. When placed under suitable culture conditions, it can enable the complete regeneration of an entire plant.

I.2. Factors influencing *in vitro* culture

The factors affecting *in vitro* regeneration can be categorized into two groups:

- Internal factors related to the plant**:** These include the genotype, the nature and ontogenetic age of the explant, as well as the physiological state of the mother plant from which the explant was taken.
- External factor: Which encompass the culture media (particularly their composition in growth regulators and sugars) and the conditions of culture establishment. **(George and De Klerk, 2008)**.

I.2.1. Effect of the explant

One of the main advantages of *in vitro* culture is that callus can produce embryos or somatic buds, whose development allows the regeneration of plants consistent with the mother plant. Various organs (buds, roots, leaves, anthers, etc.) or fragments of organs (explants) can be cultivated in a synthetic nutrient medium.

However, the in vitro response depends on numerous factors (**Saadi and Hamdani, 2007)**.

I.2.2. Physiological and ontogenetic age of the organ

Generally, in in vitro culture, it is preferable for the explants to be young (immature embryos, young leaves, meristems, etc.), as their juvenile state promotes better regeneration **(Vidalis *et al*., 1989).** The regeneration capacity of tissues from embryos is often higher and more reproducible, followed by that of cotyledons **(Saadi and Hamdani, 2007).**

I.2.3. Time of collection

Time of collection is particularly important for perennial species. There is a distinction between the active growth phase and the dormant phase of plants, leading to different in vitro responses of explants. This variation can be explained by changes in the internal balance of growth regulators (auxin, cytokinin, gibberellin, etc.) depending on the season **(Vidalis *et al*., 1989).**

I.2.4. Size of the explant

The larger the size, the more stable the endogenous balance, and the less impact external conditions will have. The ideal size depends on the type of implant. A sample intended for reproduction should be a whole organ (node, apex, or entire bud). For other types of explants, fragments of 5 to 10 mm of differentiated tissue (leaf, stem, root, inflorescence, etc.) are used **(Vidalis et al., 1989; Saadi & Hamdani, 2007).** In general, certain structures, called "target organizations," respond to morphogenetic stimuli and direct their development according to a specific program. Conversely, other structures have a low capacity to respond to in vitro conditions, mainly due to a lack of cellular plasticity **(Webb et al., 1989; Wheeler et al., 1985).**

I.2.5. Effect of genotype

Plant regeneration is often genotype-specific. Within the same species, some genotypes produce buds, while others generate only embryos **(Boxus, 1995**). Some authors indicate that only certain genotypes can induce somatic embryogenesis. In many species, this ability is under genetic control **(Vidalis et al., 1985; Isac et al., 1994; Caraglio, 2012).**

I.2.6. Culture medium

For each plant species, three types of media are distinguished: an activation medium, a multiplication medium, and a rooting medium. These media contain mineral salts, organic substances, phytohormones, and natural extracts (coconut milk, fruit juice, casein hydrolysate). Essential mineral salts are divided into two categories:

- Macro-elements (N, P, K, S, Mg, and Ca).
- Micro-elements or trace elements (B, Mn, Zn, Cu, Ni, Co, Mo, Al, I, Fe), which are equally essential, notably iron, which is crucial for growth. Various formulations have been developed, with the most commonly used being those of Murashige and Skoog, Gamborg, Knop, White, and Gautheret. **(Gamborg and Ojima, 1968).**

The mineral balance developed by **Murashige and Skoog** in **1962**, although initially designed for tobacco callus cultures, has yielded good results in many cultures but is not universal. **Gamborg and Ojima (1968)**.

In in vitro cultivated plants, photosynthesis is not essential since energy is provided by the carbohydrates in the medium. However, even in a reduced form, photosynthesis persists in the tissues. Light is crucial for triggering and properly executing certain morphogenetic processes, such as floral bud formation under long-day conditions. The required light intensity depends on the duration and spectral quality of the light received by the culture. It is measured in W.m-2 and generally ranges between 100 and 150 W.m-2 when fluorescent tubes are placed 20 cm above the culture containers. **(George and De Klerk, 2008).**

I.2.7. Temperature

Temperature is generally maintained between 20 and 25°C continuously. It is important to note that the temperature inside the culture flasks can be 2 to 4°C higher than the room temperature due to lighting. **(Kumar et al. 2009).**

I.2.8. Humidity

The relative humidity inside the culture flasks must reach 100%. However, it is essential to avoid excessive condensation on the surface of the medium, which could drown the explants. **(Lloyd, 1980).**

I.3. Plant Growth

I.3.1. Multiplication

Plant growth occurs in several stages, allowing a seed to develop into a plant capable of reproduction. This process begins with cell proliferation through mitosis, which takes place within specialized tissues called meristems. These meristems are classified as follows:

- Primary meristems: located at the root apex, the tip of stems, and in apical buds **(Vidalis *et al*., 1989).**
- Secondary meristems: found in older tissues, responsible for tissue thickening **(Saadi & Hamdani, 2007).**

Once growth is achieved, cells differentiate to perform various functions: some contribute to sap circulation (phloem and xylem), others to photosynthesis (leaves), and some to nutrient absorption (roots). This is a complex process influenced by both internal and external factors **(Boxus, 1995).**

I.3.2. Growth substances

Plant development is regulated by growth factors known as phytohormones, which exert their effects at a distance from their production site. **(Taiz et al. 2015)** These substances can act synergistically or antagonistically. The main plant hormones include:

- **Auxins**, involved in cell elongation and apical dominance **(Vidalis et al., 1989).** Auxins are a vital group of plant hormones involved in cell elongation, root initiation, and overall regulation of plant growth. In plant tissue culture, auxins are especially important due to their role in directing developmental processes. When auxins are present in higher concentrations than cytokinins, they promote root formation, guiding the explant toward root development. Auxins also play a key role in callus induction, where they stimulate the formation of a mass of undifferentiated cells—a crucial preliminary step before organogenesis. Additionally, auxins facilitate cell division and expansion by activating genes responsible for these processes and by loosening cell walls, thus enabling tissue proliferation **(Ljung, 2013).** Commonly used auxins in in vitro culture include IAA (Indole-3-acetic acid), a natural auxin; IBA (Indole-3-butyric acid); NAA (Naphthaleneacetic acid); and 2,4-D (2,4-Dichlorophenoxyacetic acid), which is particularly effective for inducing callus formation **(Dantu, 2013).**
- **Gibberellins**, composed of compounds derived from terpenes. They promote internode elongation through cell elongation and proliferation, stimulate leaf growth, and break dormancy **(Isac et al., 1994).**
- **Cytokinins**, derived from adenine and synthesized in the apex and roots. They play a crucial role in cell division **(Caraglio, 2012) .** Cytokinins are a group of plant hormones known for promoting cell division (cytokinesis), encouraging shoot proliferation, and delaying leaf senescence. In plant tissue culture, they play a complementary role to auxins, and their relative concentrations determine the developmental pathway of the explant. When cytokinins are present in higher concentrations compared to auxins, they stimulate the formation of shoots and buds. Additionally, cytokinins help overcome apical dominance by promoting the growth of lateral buds and facilitating the development of multiple shoots. Furthermore, when used in combination with auxins, cytokinins influence organogenesis and morphogenesis, guiding whether the explant develops roots or shoots, based on the hormonal balance **(Mok, 2001).**

I.3.3. Biological effects of hormonal doses

In *in vitro* cultures, two major hormones are used: **auxin** and **BAP** (a cytokinin). Their ratio determines the nature of plant growth:

- **If auxin/BAP ≈ 1**: significant callus growth **(Vidalis et al., 1985).**
- **If auxin/BAP >> 1**: preferential stimulation of root growth **(Saadi and Hamdani, 2007).**
- **If auxin/BAP << 1**: predominant development of buds and leaves **(Webb et al., 1989).**

I.4. Technical foundations of in vitro culture

In vitro culture is a technique of artificial vegetative propagation of plants that involves a set of precise methodologies. On one hand, it requires aseptic procedures such as the sterilization of equipment and the disinfection of plant material (explants). On the other hand, it depends on carefully controlled culture conditions, including specially formulated culture media tailored to each plant species, as well as regulated temperature, light, and humidity. These methods are applied to various plant organs or fragments—known as explants—which can include immature seeds, embryos, ovules, pollen, terminal or axillary buds, stem or leaf sections, flower petals, and more. For the explant to survive, multiply, and potentially regenerate into a complete plant, the culture medium must supply everything the parent plant naturally provides: mineral elements and water via the roots; sugars, vitamins, and amino acids through the leaves and photosynthesis; and plant hormones to direct organ development **(Thorpe, 2007)**. Various in vitro culture techniques are commonly used in plant biotechnology laboratories to support propagation, conservation, and genetic improvement. These include micropropagation, meristem culture, somatic embryogenesis, and haplo-diploidization through androgenesis or gynogenesis. Additionally, techniques such as embryo rescue from immature embryos—especially those resulting from interspecific crosses—are employed to ensure the viability of hybrid plants. Other advanced methods include cell culture for the production of secondary metabolites, bioencapsulation for creating artificial seeds, cryopreservation at ultra-low temperatures (−196°C), protoplast culture and somatic fusion, as well as genetic transformation. In general, in vitro plant culture follows four main stages: initiation of cultures—which is often the most delicate and critical step—followed by multiplication, rooting, and finally, weaning or acclimatization to external conditions **(Loyola-Vargas et al., 2016).**

I.4.1. The culture medium

In vitro culture success largely depends on the composition of the culture medium, which provides the essential nutrients and environmental cues needed for explant survival, growth, and regeneration. The culture medium typically consists of mineral elements, organic components, and growth regulators (hormones), each playing a distinct role in supporting plant tissue development.

I.4.2. Mineral elements

Mineral nutrients are a fundamental part of the culture medium and are classified into macroelements and microelements. Macroelements are required in large quantities and include nitrogen (N), calcium (Ca), potassium (K), sulfur (S), magnesium (Mg), and phosphorus (P). These elements are vital for the structural and metabolic functions of plant cells. On the other hand, microelements, or trace elements, are needed in much smaller quantities but are equally essential for enzymatic and physiological processes. Important microelements include iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), molybdenum (Mo), boron (B), chlorine (Cl), cobalt (Co), and nickel (Ni) **(George and De Klerk, 2008)**.

I.4.3. Organic elements

In vitro plant tissues often lack sufficient photosynthetic capability. Therefore, a source of carbon—typically in the form of sugars, most commonly sucrose—is added to the medium to support growth. In natural conditions, plants synthesize sugars via photosynthesis from atmospheric CO_2 and water from the soil, but this function must be supplemented in culture. The medium also contains vitamins, mainly from the B group, which contribute to cellular functions and development. Additionally, amino acids may be included to stimulate cell proliferation and overall culture vigor. The presence of these organic compounds has been shown to enhance the growth and regeneration capacity of cultured tissues **(Bhojwani and Razdan, 1996)**.

I.4.4. Growth regulators

Plant growth regulators, commonly referred to as phytohormones, are critical for inducing and directing the processes of growth, differentiation, and organogenesis in vitro. While plants naturally produce these substances, synthetic analogs are also widely used in tissue culture. The most frequently applied hormones are auxins and cytokinins, which are known to influence cell division, elongation, and the formation of new organs. The correct balance between these two hormones determines whether the culture favors root or shoot development. Although culture media are generally prepared in liquid form, a gelling agent (such as agar) is often added to solidify the medium and prevent explants from sinking to the bottom of the container **(Thorpe, 2007).**

I.5. In Vitro culture techniques

I.5.1. In vitro micropropagation or plant cloning

Plant reproduction can occur through sexual means, via seeds, but some species also utilize another method known as vegetative propagation. This particular form of reproduction results in daughter plants that are genetically identical to the parent plant. It is a type of plant cloning or true-to-type multiplication, a process that has been used for centuries by horticulturists and gardeners through techniques such as cutting, layering, and grafting. In vitro micropropagation follows the same principle. It involves the sterile culture of plant explants (plant fragments) on an artificial medium within a controlled environment. Through successive subcultures, it becomes possible to produce a large number of plants identical to the original one, which can be multiplied infinitely. This technique takes advantage of a fundamental property of plant cells: totipotency — their ability to regenerate an entire plant from a single cell or small group of cells (**Altman, 2010).**

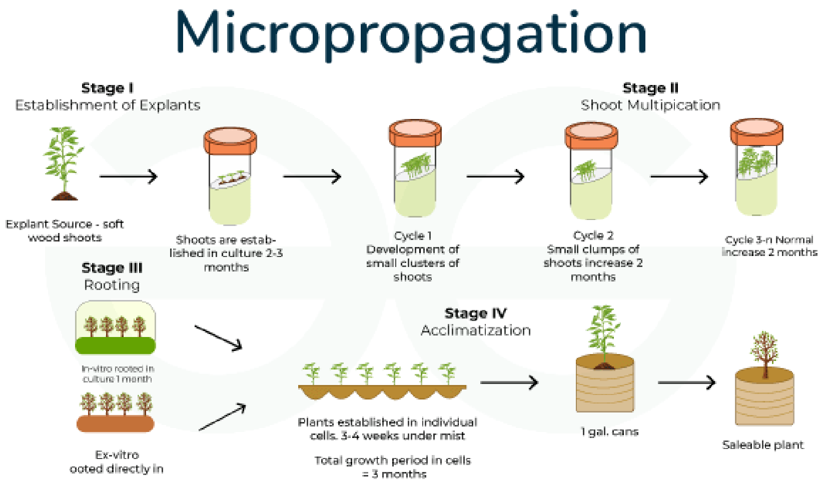

Figure 1: Overview of the micropropagation Process (**GeeksforGeeks. (2024)**

I.5.1.1. Objective and application

The main goal of in vitro micropropagation is the large-scale production of cultivars with horticultural, forestry, or agricultural value. These may be newly created or discovered varieties, or plants that continue to hold importance in plant production. The technique is also especially valuable for propagating rare or difficult-to-reproduce plants through traditional methods **(Kozai, 2016).**

I.5.1.2. Stages of micropropagation

Micropropagation is a multi-step process consisting of five main stages, each stage has its own specific objectives, requirements, and potential challenges. These stages are detailed below:

a. <u>Stage 0: Selection of an Explant</u>

This is the initial phase of the micropropagation process. It involves the careful selection of healthy stock plants, which are grown under controlled conditions prior to culture initiation (**Microbe Notes) .**

b. <u>Stage 1: Initiation of Cultures</u>

In this stage, aseptic cultures are established using suitable explants from the selected stock plants. Proper sterilization of the explants is essential for success, as contamination at this stage can compromise the entire process. This step is critical in ensuring a high success rate.(**GeeksforGeeks. 2024).**

c. Stage 2: Multiplication

This stage focuses on the rapid production of shoots. Multiplication is achieved through one of three methods: regeneration from callus, forced axillary branching, or direct adventitious bud formation from the explant. Efficient multiplication is key to the success of micropropagation.**(Plant Cell Technology 2019).**

d. Stage 3: Shoot Elongation and Rooting

While somatic embryos can develop into full plants due to the presence of both shoot and root primordia, shoots produced during Stage 2 often require additional rooting. This stage ensures the development of complete plantlets ready for transfer.(**Online Biology Notes).**

e. Stage 4: Transplantation and Acclimatization

The final stage involves transferring the plantlets from *in vitro* conditions to soil or potting mix. This is a critical phase, as plantlets often exhibit morphological, anatomical, and physiological abnormalities due to *in vitro* growth. Careful handling and gradual acclimatization are necessary to ensure survival and healthy growth. **(Grzelak, 2024).**

I.5.1.3. Methods of Micropropagation

Micropropagation encompasses several techniques that allow for the large-scale propagation of plants under controlled conditions. The choice of method depends on factors such as the plant species, the type of tissue used, and the intended goals of propagation.

a. **Meristem Culture:** This method involves isolating and cultivating meristematic tissues, typically found at the shoot tip or the base of the plant.

Meristem culture is particularly important for producing virus-free plants due to the natural absence of pathogens in these tissues. **(Preece, 1994).**

b. **Callus Method:** In this technique, plant tissues such as leaves or stem segments are cultured to induce the formation of callus—a mass of undifferentiated cells. The callus can then be stimulated to regenerate shoots and roots, resulting in the formation of multiple plantlets. **(Thorpe, 2007).**

c. **Suspension Culture Method:**This method involves culturing small tissue fragments or isolated cells in a liquid medium. It allows for the even distribution of cells and is particularly effective for large-scale propagation and biochemical studies. **(Sharma, 2018).**

d. **Embryo Culture:** Embryo culture consists of isolating immature embryos from seeds and cultivating them *in vitro* to develop into complete plants. This technique is commonly used in the propagation of difficult species such as orchids. **(Kao, 1979).**

e. **Protoplast Culture:** Protoplasts are plant cells that have had their cell walls enzymatically removed. Once isolated, these cells are cultured in a suitable medium, where they regenerate cell walls and eventually develop into full plants. This method is useful for plant species where conventional propagation is challenging. **(Davey, 1989).**

I.5.1.4. Advantages and limitations of in vitro micropropagation

In vitro micropropagation offers numerous advantages that make it a powerful tool in plant biotechnology, horticulture, and conservation. One of the primary benefits is the ability to produce genetically identical plants that are true-to-type clones of the parent plant or variety. This genetic uniformity ensures consistency in performance, which is especially valuable in commercial agriculture. Additionally, the resulting plants are often of excellent sanitary quality, exhibiting vigorous growth with well-developed roots and abundant branching, due to their production under aseptic conditions. **(De Klerk, 2008).**

Another significant advantage is the acceleration of plant production cycles. Micropropagation allows for the rapid generation of large quantities of plantlets, facilitating quicker market introduction of new cultivars. Because this technique is independent of seasonal fluctuations, it allows for year-round production and more efficient use of greenhouse space. Moreover, the technique reduces the need for a large number of mother plants and eliminates the necessity of rootstocks in some fruit and ornamental species, thereby conserving space and resources. **(Kumar, 2024).** Micropropagation also provides solutions for species that are traditionally difficult to propagate. For example, it enables the cost-effective cultivation of complex species like orchids by eliminating the need for symbiotic fungi during germination. Sterile plants, which are unable to reproduce naturally, can also be successfully propagated through in vitro culture. This technique plays an essential role in the conservation of old or rare varieties, which can be maintained in a miniaturized, pest- and disease-free environment, allowing more than 1,000 plantlets to be preserved per square meter. Additionally, in vitro propagation supports rapid reforestation efforts by enabling the mass production of plants for replanting in areas affected by pests or natural disasters. The aseptic nature of vitroplants also facilitates safe international transport, as they pose minimal phytosanitary risk during shipment **(Bhat, 2018)**.

Despite its many benefits, in vitro micropropagation is not without limitations. The primary disadvantage is its relatively high production cost compared to conventional propagation methods. This is largely due to the requirement for sterile environments, controlled growth conditions, and the use of culture media. Furthermore, the technique necessitates a skilled workforce, with labor accounting for approximately 60% to 70% of total production expenses **(Cassells, 2001).**

I.5.2. Meristem and Bud culture

Meristems are zones of intensely dividing cells, located at the heart of buds and at the tips of roots, and are responsible for the formation of leafy stems or the root system.

In 1950, the work of Limasset and Cornuet demonstrated that meristems are free of viruses. Meristem culture is an aseptic culture on an artificial medium of the apical dome without leaf primordia.It measures 0.2 to 0.3 mm across and dissection is performed under a binocular magnifying glass. The technique can be combined with thermotherapy: culture at elevated temperatures to promote virus elimination **(Wang al., 2018).** Many plant varieties from different species have been preserved using this technique. For example, it helped save potatoes like 'Belle de Fontenay' as early as 1954, as well as dahlias, strawberries, grapevines, irises, raspberries, and more. More recently, the Violette de Toulouse was rescued from decline through meristem culture, which made it possible to regenerate healthy, virus-free plants **(Benmahioul, 2020)**.

According to **Benmahioul (2020),** the in vitro propagation process through meristem and bud culture involves several key steps. First, a healthy, vigorous mother plant free from viruses and visible diseases is selected. Under a binocular microscope with cold light to prevent desiccation, apical meristems measuring 0.2 to 0.5 mm are carefully isolated. These explants are then prepared with meticulous handling to minimize contamination risks. The explants are cultured on a nutrient-rich medium, typically Murashige and Skoog (MS) medium, supplemented with auxins and cytokinins to promote growth. Optionally, thermotherapy may be applied to eliminate potential viruses by exposing the explants to controlled heat. The cultures are then incubated under optimal conditions of temperature and light. Sanitary status is verified through virus detection methods, including biological indexing on sensitive indicator plants and serological tests such as ELISA or Dot-ELISA. **(Wang, 2018).** Once meristems have developed, they are transferred to a suitable medium for rooting and further growth. Acclimatization follows, where plantlets are gradually adapted to external conditions by slowly reducing relative humidity, increasing exposure to natural light, and maintaining appropriate temperature and ventilation. This phase helps plantlets develop functional cuticles, active stomata, and improved environmental stress tolerance. Finally, fully acclimatized plantlets are transplanted into horticultural substrates such as peat, perlite, or vermiculite, and cultivated in pots or directly in the soil depending on the species and intended use, with appropriate watering and fertilization.

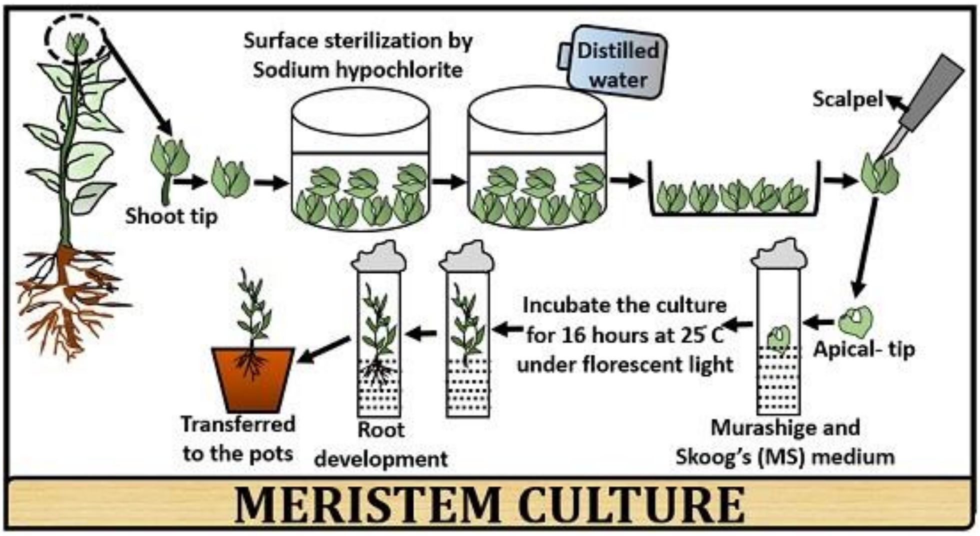

Figure 2 Diagram of Meristem Culture Technique **(*Biology Reader.2025)***

I.5.2.1. Process steps

The process of meristem culture involves several critical steps to ensure the successful regeneration of healthy, virus-free plants. It begins with the selection of the mother plant**,** where a vigorous, disease-free specimen is chosen to serve as the source of explants. Next, the isolation of meristems is carried out under a binocular microscope using cold light to prevent desiccation. Apical meristems, typically measuring between 0.2 to 0.5 mm, are carefully extracted during this step. Following isolation, the preparation of explants is performed with utmost caution to minimize the risk of microbial contamination. The prepared meristems are then cultured on a nutrient-rich Murashige and Skoog (MS) medium**,** which is supplemented with specific concentrations of auxins and cytokinins to stimulate cellular growth and development.

In some cases, thermotherapy may be applied as an optional but effective step. This involves exposing plant material to controlled heat treatments to help eliminate latent viral infections. After placement on the culture medium, incubation takes place under strictly regulated environmental conditions, including optimal light and temperature, to promote growth.

To verify the sanitary status of regenerated tissues, virus detection is conducted using both biological indexing—where explants are grafted onto sensitive indicator plants—and serological tests such as ELISA or Dot-ELISA.

Once meristems develop into plantlets, the regeneration phase involves transferring them to a suitable medium that supports root formation and shoot elongation. This is followed by acclimatization, a gradual adaptation of the plantlets to external environmental conditions. Key steps during this phase include the progressive reduction of relative humidity, the controlled increase of natural light exposure, and the maintenance of appropriate temperature and ventilation. These measures help in the development of a functional cuticle, active stomata, and enhanced resistance to environmental stress. Finally, after successful acclimatization**,** transplantation is performed. The plantlets are transferred to horticultural substrates such as peat, perlite, or vermiculite, depending on species-specific requirements and propagation goals. At this stage, ensuring adequate irrigation and fertilization is essential to support continued growth in either pots or open soil (**Benmahioul, 2020)**.

I.5.2.2. Objective and application

Meristem culture is the only reliable method for producing healthy, virus-free plants. Since viruses typically do not infect the meristematic tissue at the growing tips of plants, isolating and culturing these cells allows for the regeneration of disease-free individuals, making this technique essential for the sanitary improvement and preservation of valuable plant varieties.**(Wang, (2008).**

I.5.2.3. Advantages and limitations of meristem and bud culture

Meristem culture enables the recovery of plant varieties threatened with extinction due to severe viral infections. It is particularly applied to vegetatively propagated species—such as Pelargonium, dahlia, chrysanthemum, potato, artichoke, strawberry, and raspberry—since these propagation methods often transmit viruses to the progeny. The technique produces healthy plants that are free from viruses, fungi, and bacteria, and that meet the increasingly strict international phytosanitary standards. These sanitized plants show improved vigor and the restoration of their flowering and fruiting capacities. The resulting individuals remain true to the original variety and can be multiplied in large numbers, ensuring uniform and high-quality crops (Wang, 2018). However, meristem culture has its limitations: while it eliminates viruses, it does not confer virus resistance, leaving plants vulnerable to reinfection through insect vectors if proper preventive measures are not taken. Moreover, for varieties with chimeric traits—such as variegated Petunia or Pelargonium—these distinctive features may be lost in the regenerated plants (Thorpe, 2007).

I.5.3. Somatic embryogenesis

Somatic embryogenesis is a form of vegetative propagation that allows the production of numerous plantlets that are genetically identical to the donor plant of the explants. It refers to the development of embryos from somatic cells, i.e., non-sexual cells. **(Jain, 2010).** Numerous cell divisions are rapidly induced from the cultured tissues thanks to the application of a high dose of auxin. A callus is then formed—this is a mass of undifferentiated, rejuvenated cells that can give rise to bipolar embryos, which will behave like zygotic embryos (resulting from the fertilization between a female and a male sex cell) **(Bairu and Van Staden, 2013)**.

I.5.3.1. Objective and application

Somatic embryogenesis is a technique particularly well-suited for industrial-scale plant production. It involves the development of embryos from somatic (non-reproductive) cells and can be carried out in bioreactors. This allows for the large-scale production of somatic embryos, which can either be encapsulated in a nutritive gel to form artificial seeds or used for the synthesis of valuable secondary metabolites, such as those employed in medicine and natural dyes. Somatic embryogenesis also serves as an effective method for cloning woody plants. A single liter of culture medium in a fermenter can yield thousands or even millions of somatic embryos. This technique is applicable to a wide range of species, including fennel, coffee, lemon, alfalfa, carrot, petunia, eggplant, cocoa, rubber tree, and oil palm **(Ramesh and Suresh, 2011)**.

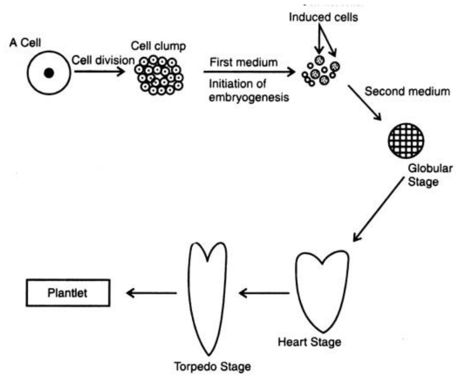

Figure 3 Events of somatic embryogenesis **(Ammirato, 1983)**

I.5.3.2. Advantages and limitations of Somatic embryogenesis

This method of propagation can be automated, which helps lower production costs. It produces a very high number of plants. Since the embryos come from a single cell, there is no problem with chimeras (plants with mixed genetic traits). Somatic embryogenesis also helps renew plant tissues and makes it easier to introduce genetic changes. It is a good way to clone high-quality woody plants **(Wang and Jin, 2013)**. However, because the process involves a callus stage (a mass of cells), there is a risk that some plants may develop genetic differences from the original **(Loyola-Vargas and Ochoa-Alejo, 2016)**.

I.5.4. Haplo-Diploidization technique

Haplo-diploidization is a biotechnological method that enables the rapid production of genetically pure plant lines. It involves two main steps. First, haploid cells are obtained by culturing male or female reproductive organs—such as anthers or isolated pollen grains—in vitro, where the cells are reprogrammed to develop embryogenic potential. These haploid cells, which contain only one set of chromosomes, are then subjected to chromosome doubling, often using agents like colchicine. This results in diploid plants that are completely homozygous. Haploid plants can be produced through androgenesis (from male gametes) or gynogenesis (from female gametes) **(Guo and Zhao, 2008)**.

I.5.4.1. Objective and application

The goal of haplo-diploidization is to generate homozygous lines in a single generation, bypassing the need for multiple cycles of self-fertilization. This makes the method highly valuable for plant breeding and genetic improvement programs, where stable and uniform lines are essential**. (Mishra, 2022).**

I.5.4.2. Haploid plant production through androgenesis and gynogenesis

Haploid plant production can be achieved through two main techniques: androgenesis and gynogenesis. Androgenesis involves the development of haploid plants from male gametophytes through the culture of anthers or isolated microspores. In contrast, gynogenesis refers to the regeneration of plants from the culture of unfertilized female reproductive organs, such as immature ovules or ovaries, grown on artificial media. In both cases, the resulting plantlets contain only one set of chromosomes. To make these plants fertile and genetically stable, chromosome doubling is induced using chemical agents like colchicine, which blocks mitosis after chromosome replication. This approach allows for the rapid creation of completely homozygous lines within a few weeks, a process that would otherwise take 8 to 10 years through conventional self-fertilization methods **(Dulai and Jain, 2009)**.

I.5.4.3. Advantages and limitations of the Haplo-Diploidization technique

Haplo-diploidization offers several advantages, making it a valuable tool in plant breeding programs. It enables the rapid production of completely homozygous lines in a single generation, thus saving years of self-fertilization typically needed to achieve genetic purity. The resulting plants are genetically uniform and stable, which is ideal for both large-scale propagation and genetic studies. This technique also facilitates mutation analysis and genetic transformation experiments, and it can be applied to a wide range of plant species through androgenesis or gynogenesis. However, the method has certain limitations. Its efficiency largely depends on the plant species and genotype, as some are more responsive to in vitro culture than others. The process can be technically demanding, requiring precise culture conditions and skilled manipulation, especially during the chromosome doubling stage. Moreover, if a callus phase occurs, there is a risk of somaclonal variation, which can lead to unwanted genetic changes. Additionally, haploid plants are initially sterile and must undergo chromosome doubling to become fertile and useful for breeding purposes **(Ferreira, 2023).**

CHAPTER TWO

MEDICINAL PLANTS

II.1. Medicinal plants

Medicinal plants are botanical species recognized for their therapeutic potential, primarily due to the presence of natural bioactive compounds such as alkaloids, flavonoids, terpenoids, and phenolics. These compounds exert a wide range of pharmacological effects, enabling the use of medicinal plants in the prevention, management, or treatment of numerous human and animal diseases. As a result, they play a significant role in both traditional healing systems and modern pharmaceutical research **(Heinrich, 2012).**

The use of medicinal plants dates back more than 5,000 years, with early evidence found in ancient civilizations such as Egypt, China, India, and among indigenous peoples. Historical texts like the Egyptian Ebers Papyrus and Indian Ayurvedic treatises highlight the longstanding importance of herbal medicine **(Sofowora, 1993).** Medicinal plants exert their effects through active compounds such as alkaloids, flavonoids, tannins, and essential oils, which vary depending on the species and the part of the plant used (root, leaf, flower, bark, etc.). These substances contribute to various therapeutic effects, including anti-inflammatory, antimicrobial, digestive, and calming actions **(Chevallier, 2016)**.

Medicinal plants are commonly classified into several categories: digestive plants (e.g., peppermint, ginger, chamomile), calming plants (e.g., valerian, passionflower, lavender), antiseptic plants (e.g., thyme, eucalyptus, oregano), anti-inflammatory plants (e.g., turmeric, meadowsweet, white willow), and immunostimulant plants (e.g., echinacea, ginseng, garlic) **(Chevallier, 2016)**.

Medicinal plants can be used in various forms depending on their intended purpose and the part of the plant utilized. Common methods include infusions and decoctions, such as chamomile tea for treating insomnia, as well as concentrate extracts like mother tinctures. Essential oils—for example, lavender essential oil—are widely used for conditions such as anxiety. Other forms include poultices, such as cabbage leaves applied to reduce inflammation, and dietary supplements in the form of capsules or powders **(Bone and Mills, 2013)**. The advantages of using medicinal plants include their natural origin, which makes them generally gentler than synthetic drugs, their accessibility—particularly in rural or traditional cultures—their affordability, and their alignment with ecological and cultural values **(WHO, 2002)**.

Table 01 : Examples of common medicinal plants and their benefits (Radiant Psyche, 2025).

Plant	Main Use	Method of Use
Aloe Vera	Burns treatment	Apply gel directly to the skin
Chamomile	Relaxation, digestion	Tea after meals
Peppermint	Anti-nausea, headache	Tea or essential oil
Turmeric	Anti-inflammatory	Powder in food
Lavender	Anxiety, insomnia	Aromatherapy or tea
Garlic	Cardiovascular health, antimicrobial	Raw, cooked, or in capsules
Ginger	Anti-nausea, digestion	Fresh, tea, or powder
Thyme	Respiratory issues, antiseptic	Tea or essential oil
Basil (Holy Basil / Tulsi)	Stress relief, respiratory issues	Tea or fresh leaves

II.1.1. Secondary metabolites from medicinal plants

Medicinal plants are rich sources of bioactive compounds that contribute to their therapeutic effects. These natural substances, produced through secondary metabolism, include a variety of chemical groups such as polyphenols, alkaloids, terpenoids, and essential oils, each with specific biological activities. Among them, polyphenols are particularly well-studied for their health benefits.

II.1.2. Polyphenols

Polyphenols are a diverse group of naturally occurring compounds found in plants, widely recognized for their antioxidant properties. They are produced as a result of secondary metabolism in plants and play a significant role in plant defens mechanisms, such as protecting against pathogens and UV radiation. **(Pandey, 2009).** Polyphenols can be classified into several major types, each contributing uniquely to human health. Flavonoids are the most common group, found abundantly in fruits, vegetables, and grains. Compounds such as quercetin, catechins, and anthocyanins within this group are known for their strong antioxidant properties. Phenolic acids, including tannins and lignans, are present in foods like berries, grains, and seeds, and contribute to the plant's defense and potential health benefits. Another well-known polyphenol is resveratrol, found in grapes and red wine, which has been associated with anti-inflammatory and anti-cancer effects (Manach et al., 2004). The health-promoting functions of polyphenols are numerous. Their antioxidant activity allows them to neutralize free radicals, helping to prevent cell damage and reduce the risk of chronic diseases such as cardiovascular disorders and cancer. Additionally, polyphenols possess anti-inflammatory properties that support immune function and can aid in the management of inflammatory conditions like arthritis. Some polyphenols have also demonstrated the ability to interfere with cancer development and progression. Moreover, compounds like resveratrol have been linked to cardiovascular health by enhancing blood circulation and reducing cholesterol levels **(Fraga et al., 2019).**

II.1.3. Terpenes

Terpenes are a large and diverse class of organic compounds produced by plants and are often the primary constituents of essential oils. They play a crucial role in contributing to the aroma, flavor, and color of many plants, while also serving as natural defense mechanisms against herbivores, insects, and pathogens (Russo, 2011). Terpenes are categorized into several types based on their structure and carbon count. Monoterpenes ($C_{10}H_{16}$), such as limonene in citrus oils and pinene in pine trees, are the simplest form. Sesquiterpenes, found in essential oils like ginger and sandalwood, exhibit a wide range of biological activities. Diterpenes include compounds like taxol, an anticancer drug derived from yew trees. Triterpenes and saponins are significant for plant defense and are widely used in medicine for their anti-inflammatory and anti-tumor effects (Gershenzon, 2007). Beyond their biological roles in plants, terpenes offer various health benefits. Many, like pinene and limonene, possess natural antibacterial and antifungal properties. Others, such as beta-caryophyllene—found in black pepper and cloves—exhibit anti-inflammatory and analgesic effects. Terpenes are also integral to aromatherapy, promoting relaxation and reducing anxiety; for instance, linalool in lavender oil is known for its calming effects. Additionally, certain terpenes, particularly those in citrus fruits, have antioxidant properties that help combat oxidative stress and reduce the risk of related diseases **(Russo, 2011)**.

II.1.4. Essential oils

Essential oils are concentrated plant extracts composed primarily of terpenes, phenolics, and other secondary metabolites. They are produced in specialized plant structures such as glandular cells, resin ducts, and trichomes, and are known for their strong aromatic properties, which make them valuable in perfumes, cosmetics, and herbal remedies **(Bakkali et al., 2008)**. Various essential oils contain bioactive compounds responsible for their therapeutic effects. For example, lavender oil contains linalool and linalyl acetate, which have calming and stress-relieving properties. Peppermint oil, rich in menthol and menthone, is known for its cooling and soothing effects and is commonly used for digestive issues and headaches. Eucalyptus oil, which contains eucalyptol, is often employed in treating respiratory conditions due to its antimicrobial and anti-inflammatory actions **(Sharifi-Rad et al., 2017).** Essential oils offer a wide range of health benefits. Many, such as tea tree, thyme, and oregano oils, possess strong antibacterial, antiviral, and antifungal properties. Oils like lavender, chamomile, and bergamot are frequently used in aromatherapy for stress relief and mood stabilization. Additionally, essential oils such as eucalyptus and peppermint are used for pain management due to their anti-inflammatory and analgesic effects. In skincare, oils like tea tree and lavender are popular for treating acne and promoting skin healing, thanks to their antimicrobial and regenerative properties **(Fakhari and Dastgheib, 2019).**

II.2. Biological role of secondary metabolites

Secondary metabolites, including polyphenols, terpenes, and essential oils, are specialized compounds that plants produce which do not directly participate in their fundamental metabolic functions such as growth, development, and reproduction. Instead, these compounds serve essential ecological and physiological roles that are crucial for the plant's survival and adaptation to its environment. One of their primary functions is to protect plants from a variety of biotic threats, including herbivores and pathogenic microorganisms, by acting as natural deterrents or toxins. Additionally, these metabolites play a significant role in facilitating reproduction by attracting pollinators through their distinct fragrances, colors, and flavors, thereby ensuring successful pollination and the continuation of the species. Beyond these roles, secondary metabolites function as allelopathic agents, releasing chemicals into the environment that inhibit the germination and growth of competing plant species nearby, which reduces competition for resources such as light, water, and nutrients. Moreover, these compounds enhance the plant's resilience against abiotic stresses by providing protection against damaging environmental factors like ultraviolet (UV) radiation, extreme temperature fluctuations, and prolonged drought conditions. This multifaceted contribution of secondary metabolites not only supports individual plant health and survival but also influences ecological interactions and biodiversity within plant communities, underscoring their importance in both plant biology and ecosystem dynamics **(Yadav, 2010).**

CHAPTER THREE APPLICATIONS OF IN VITRO CULTURE FOR MEDICINAL PLANTS

III.1. In vitro culture of medicinal plants

Plant tissue culture, also known as in vitro cell culture or sterile culture media, plays a significant role in both fundamental and applied studies. This term mainly refers to the in vitro culture of plant cells, tissues, and organs. Today, the applications of plant tissue culture focus primarily on clonal propagation, and routine technologies have expanded to include somatic embryogenesis, somatic hybridization, virus elimination, and the use of bioreactors for mass propagation of plants **(Gaurav et al., 2018)**.At the same time, the richness of medicinal plants is under threat due to rapid loss, with many valuable medicinal plants at risk of extinction because of the fast depletion of natural resources. Pharmaceutical companies largely depend on materials extracted from wild plants, which are becoming increasingly scarce. In this context, plant tissue culture represents an alternative method for commercial propagation, widely used for the multiplication of many plant species, including a large number of medicinal plants. The traditional use of medicinal plants for therapeutic purposes in humans has also been well documented **(Shimomura et al., 1997)**.

In vitro culture is a widely used technique in the field of medicinal plants, offering a reliable and controlled method for plant propagation and the production of valuable bioactive compounds. This biotechnological approach enables the rapid multiplication of plant species with therapeutic potential, ensuring the conservation of rare or endangered medicinal plants while also allowing for year-round production regardless of seasonal changes. Furthermore, in vitro culture plays a crucial role in enhancing the production of secondary metabolites—such as alkaloids, flavonoids, and terpenoids—which are commonly used in the pharmaceutical, cosmetic, and nutraceutical industries. As a result, it has become an essential tool in modern research and industrial applications related to medicinal plants **(Debnath, 2017).**

III.2. Applications of in vitro culture for medicinal plants

III.2.1. Production of essential oils

The term "essential oil" or "plant essence" refers to a complex mixture of hydrophobic and volatile liquid compounds derived from plants, characterized by a strong specific aroma. According to the French Agency for Standardization (AFNOR NT 75-006), an essential oil is defined as: "a product obtained from a plant raw material, either by steam distillation, mechanical processes applied to the peel of citrus fruits, or dry distillation, and separated from the aqueous phase by physical methods" **(Bakkali, 2008)** Essential oils are found only in a limited number of plants, about 10% of higher plants. It is estimated that around fifty plant families, comprising approximately 17,500 species, produce these secondary metabolites. The synthesis and accumulation of these metabolites in an organ are associated with the presence of specialized histological structures, which may be secretory cells, secretory pockets, glandular hairs, or secretory canals, depending on the botanical species. These essential oils are present in various plant organs such as flowers (e.g., rose), leaves (e.g., lemongrass), roots (e.g., iris), bark (e.g., cinnamon), rhizomes (e.g., ginger), bulbs (e.g., garlic), wood (e.g., juniper), fruits (e.g., vanilla), and seeds (e.g., nutmeg) **(Nebie, 2023).**

Table 2: Examples of medicinal plants producing essential oils

Species	Family	Plant Origin	Culture Medium	Type of Culture	Culture Conditions	Produced Constituents	Reference
Ocimum basilicum	Lamiaceae	Seeds	MS	Micropropagation	Temperature: 27°C ±1, Light intensity: 6000 lux, Relative humidity: 80%, Photoperiod: 12 h/day	Monoterpenes: γ-Terpinene, Ortho-cymene, α-Terpinolene, Terpinen-4-ol, Linalool Sesquiterpenes: Caryophyllene, Trans-α-bergamotene	**Dossoukpévi et al. (2016)**
Perilla frutescens var. crispa	Lamiaceae	Japan	MS + 2,4-D (1.0 ppm), Kinetin (5.0 ppm)	Callogenesis	30 g sucrose/liter, 0.9% w/v agar	Isolation and identification of monoterpenes (limonene)	**Sugisawa & Ohnishi (1976)**
Calypogeia granulata	Calypogeiaceae	Japan	MSK-4 medium + 2,4-D	Suspension culture	2% glucose	Isolation and determination of sesquiterpenes	**Takeda & Katoh (1981)**
Valeriana officinalis	Caprifoliaceae	Belgium	Solid medium	In vitro cultures of seed	No details provided	Essential oil isolation	**Violon & Sonck (1984)**
Centranthus macrosiphon	Caprifoliaceae	Belgium	Solid medium	In vitro cultures of seed	No details provided	Essential oil isolation	**Violon & Sonck (1984)**

III.2.2. Production of bioactive compounds

In vitro culture (IVC) is an essential method for the sustainable and controlled production of bioactive compounds in medicinal plants. These compounds play an important role due to their therapeutic properties, such as antioxidants, flavonoids, alkaloids, and saponins. Natural bioactive compounds present in plants and foods constitute a rich source of molecules used in the manufacture of dietary supplements, functional foods, and food additives. These compounds are characterized by a great diversity in structure and function, with some present at high concentrations, such as polyphenols. Moreover, several plants have been cultivated in the laboratory to increase the production of these bioactive compounds for the extraction of active elements. **(Debnath, S. C. 2017).**

Table 3: Examples of antioxidant production through in vitro culture in medicinal plants

Medicinal Plant	Exploited Part	Culture Medium	Bioactive Compounds Produced	Reference
Rosmarinus officinalis	Apex (apical meristems)	MS + BA (1 mg/L) + NAA (0.5 mg/L)	Rosmarinic acid, flavonoids	**Căchiță-Cosma et al., 2004; Gautheret, 1959; Murashige & Skoog, 1962; White, 1954**
Ginkgo biloba	Intact embryos, embryos without cotyledons, excised cotyledons	Minimal organic MS medium supplemented with sucrose, agar, and various hormones (2,4-D, NAA, BA, Kinetin)	Ginkgolides A and B	**Camper et al., 1995**
Curcuma longa	Rhizomes	MS medium enriched with vitamins, hormones (BAP, NAA), sucrose, agar; temporary immersion system (TIS)	Curcumin, essential oils, antioxidants	**Marchant et al., 2021**
Withania somnifera	Roots	MS + vitamin B5 + BAP, Kinetin (KN), NAA, IBA	Tropane alkaloids, withanolides, steroidal lactones	**Ramesh et al., 2022**
Camellia sinensis	Leaf (callus)	MS enriched with growth regulators	Polyphenon	**Sutini et al., 2018**
Ocimum sanctum	Leaves	MS + Kinetin (1 mg/L) + IAA (0.2 mg/L)	Eugenol, flavonoids	**Manjudevi et al., 2022**
Aloe vera	Stem cuttings	MS + BA (2 mg/L) + NAA (0.2 mg/L)	Anthraquinones, aloin	**Ahmed et al., 2007**
Zingiber officinale	Induced buds from rhizomes	MS + BAP; Half-strength B5 + NAA + GA_3	Gingerols, shogaols	**Mohamed et al., 2011**
Echinacea purpurea	Aerial seeds	MS + 2,4-D (1 mg/L) + Kinetin (0.5 mg/L)	Alkamides, caffeic acid	**Zayova et al., 2012**
Phyllanthus amarus	Suspension cells	MS + NAA (1 mg/L) + Sucrose (30 g/L)	Lignans, flavonoids	**Xavier et al., 2012**

III.2.3. Phytoremediation and In vitro culture

Phytoremediation is a sustainable environmental technique that uses plants to remove, neutralize, or stabilize pollutants in the environment, such as heavy metals, organic chemicals, and industrial contaminants. This approach holds great potential for managing soil and water pollution, offering a more economical and eco-friendly alternative to traditional cleanup methods **(Pardo et al., 2013; Salt et al., 1998**). However, to ensure maximum efficiency of this technique, it is crucial to select plants capable of optimally tolerating and absorbing these pollutants **(Rascio et al., 2010).**

In vitro culture plays a fundamental role in the improvement and selection of plants adapted to phytoremediation. Through this technique, it is possible to stimulate plant growth and induce mutations that enhance their ability to absorb pollutants **(Siddiqui et al., 2015).** Factors such as growth regulators or elicitors can be used to activate the natural detoxification mechanisms of plants **(Yadav et al., 2011).** Moreover, in vitro culture offers a controlled environment that allows for accelerated selection processes and the production of uniform plants, better suited for the remediation of contaminated soils **(Khan et al., 2012).** Thus, integrating phytoremediation with in vitro culture techniques constitutes an innovative approach for more effectively treating polluted sites. By enhancing plants' ability to absorb heavy metals and other pollutants, this method contributes to the restoration of degraded ecosystems and to the reduction of environmental risks for humans and wildlife **(Salt et al., 1998; Raskin et al., 1997).**

Table 4: Examples of plants used in phytoremediation via in vitro culture

Medicinal Plant	Target Pollutant	In Vitro Culture Strategy	Reference
Brassica juncea	Heavy metals (Pb, Cd, Zn)	Root culture in MS medium enriched with jasmonic acid	**Nehnevajova et al., 2007**
Hypericum perforatum	Environmental conditions, pollutants, fungi, bacteria, viruses, insects	Callus induction and in vitro regeneration using growth regulators (BA, Kinetin, 2,4-D, IBA) on MS medium	**Parsamanesh et al., 2018**
Phragmites australis	Industrial and domestic wastewater pollutants	Callogenesis and selection of resistant lines	**Lee et al., 2006**
Salix alba	Heavy metals	Optimized hydroponic culture	**Bousbih et al., 2023**

III.3. Conservation and multiplication of rare medicinal plants

In *vitro* culture techniques play a key role in various fields, notably in crop improvement and the conservation of rare medicinal plants. These methods offer a sustainable solution for preserving endangered plant species while responding to the growing demand for natural products. In fact, in vitro culture allows for the rapid multiplication of healthy and genetically stable plants under controlled conditions, regardless of seasons and environmental factors.This approach thus guarantees a constant supply, with high yields, for industries dependent on medicinal plants **(Rodriguez et al., 2016).**

In the context of conserving rare medicinal plants, in vitro culture allows for the multiplication of these species without compromising their natural habitats. Furthermore, this technique offers the possibility of producing large quantities of genetically identical plants, which is crucial for the pharmaceutical and cosmetic industries. By using techniques such as micropropagation, cell suspension culture, and somatic embryogenesis, researchers manage to maintain the genetic diversity of plants while optimizing their yield of active compounds These techniques ensure stable and continuous production of high-quality plants for therapeutic treatments. **(Kim et al., 2018; Jia et al., 2008; Abdellatif et al., 2018).** Thus, in vitro culture constitutes an essential method for the conservation and multiplication of rare medicinal plants, allowing the preservation of these natural resources while supporting their sustainable exploitation. These methods contribute not only to the conservation of endangered species but also to the production of high-quality medicinal plants for various industrial applications **(Pokhrel et al., 2010).**

Table 5: Examples of In vitro conservation and multiplication of medicinal plants

Medicinal Plant	Organ Used	Culture Medium	Multiplication Method	Reference
Artemisia annua	Cuttings	MS + BA (1.5 mg/L) + NAA (0.5 mg/L)	Micropropagation	**Rodriguez et al., 2016**
Panax ginseng	Roots	B5 + Sucrose (30 g/L)	Cell suspension culture	**Kim et al., 2018**
Saussurea involucrata	Callus	WPM + 2,4-D (1 mg/L) + Kinetin (0.5 mg/L)	Somatic embryogenesis	**Jia et al., 2008**
Taxus baccata	Young shoots, callus	B5, then MS with hormones	Cell suspension, synchronization, elicitation	**Abdellatif et al., 2018**

III.4. Economic Importance of medicinal plants cultivated In Vitro

The in vitro cultivation of medicinal plants presents significant opportunities to boost exports and enhance global market competitiveness. These lab-produced plants are considered high-value products that allow countries to fully leverage their native botanical resources, developing export-ready health and cosmetic products that comply with stringent international quality standards. This not only strengthens national economies but also positions these countries favorably in the global marketplace. In addition, the rise of medicinal plant biotechnology fosters the development of a specialized industry. Establishing dedicated laboratories for plant production supports agricultural biotechnology startups and creates new employment opportunities in fields such as plant science, pharmacy, and bioengineering. This industry growth contributes to innovation and economic diversification. **(Bhojwani.. 2013).**

Furthermore, lab cultivation of medicinal plants plays a crucial role in environmental conservation. It helps reduce pressure on wild plant populations by protecting endangered species, preserving biodiversity, and promoting the sustainable use of natural resources. By growing plants under controlled conditions, overharvesting from natural habitats can be minimized. Countries with rich botanical diversity, such as Algeria, India, and Morocco, stand to gain substantial benefits from in vitro cultivation. These nations can export high-quality, lab-grown medicinal plant products, earn valuable foreign currency, and secure contracts with global pharmaceutical companies. Moreover, adherence to international standards like WHO-GMP (Good Manufacturing Practices) is more easily achieved through controlled laboratory production, further enhancing export potential and product reliability . **(Goyal.. 2011).**

Medicinal plants have long been valued for their healing properties, thanks to bioactive compounds like polyphenols, terpenes, and essential oils. These secondary metabolites not only provide health benefits but also help plants defend against stress and pests. With rising demand for natural remedies, in vitro cultivation offers a sustainable way to produce high-quality, disease-free plants while protecting wild populations. This technology supports biotechnology industries, boosts exports, and promotes economic growth by meeting international standards. Combining traditional knowledge with modern science, in vitro propagation holds great promise for the future of medicinal plant use. **(Ramawat.. 2008).**

CONCLUSION AND PERSPECTIVES

Conclusion and perspectives

This study has clearly demonstrated that in vitro cultivation of medicinal plants constitutes a strategic approach for the production of high-value secondary metabolites from various plant tissues, including leaves, stems, roots, and buds. Beyond merely replicating known compounds, in vitro techniques offer the potential to discover novel bioactive molecules absent in the parent plants, thereby opening new avenues for pharmaceutical research and enabling large-scale production of therapeutic compounds, often at higher yields than those obtained through conventional cultivation. Moreover, in vitro propagation plays a vital role in the conservation and mass multiplication of medicinal plants that are difficult to propagate naturally or are at risk of extinction. It supports biodiversity conservation efforts and the rehabilitation of endangered natural populations. This technique also facilitates the production of virus-free, genetically uniform plants with enhanced agricultural and medicinal traits, meeting the demands of both agricultural production and the pharmaceutical industry. Micropropagation methods, particularly the use of shoot tip and nodal cultures, have been among the most widely adopted and successful strategies for preserving the genetic integrity of elite medicinal plant species. These methods have significantly contributed to satisfying the increasing global demand for natural products used in pharmaceuticals and cosmetics. However, variations in in vitro culture requirements among plant species underscore the ongoing need to develop and standardize reliable propagation protocols for each species. **(Atanasov.. 2015).**

The findings of this study underscore that in vitro cultivation represents a major advancement not only in agricultural biotechnology but also in scientific research. While such technologies illustrate the growing ability of humans to manipulate natural systems, they also prompt important ethical and scientific considerations regarding the extent and implications of this control. Despite its promising potential, the successful implementation of in vitro cultivation requires stringent aseptic conditions and highly trained technical staff possessing meticulous skills and scientific rigor. Furthermore, in vitro flowering techniques, which hold significant promise for producing self-fertilized seeds and enhancing genetic selection, remain underexplored in many plant species, including medicinal plants. Continued research and the development of species-specific protocols are essential to fully realize the potential of these biotechnological approaches. **(Bhojwani.. 2013).**

BIBLIOGRAPHIC REFERENCES

Bibliographic references

Altman, A., & Loberant, B. (2010). Micropropagation: Clonal plant propagation in vitro. In A. Altman & P. M. Hasegawa (Eds.), *Plant biotechnology and agriculture: Prospects for the 21st century* (pp. 1–17). Academic Press. https://doi.org/10.1016/B978-0-12-381466-1.00001-2

Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P., ,. & Stuppner, H. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances*, *33*(8), 1582–1614. https://doi.org/10.1016/j.biotechadv.2015.08.001

Ammirato, P. V. (1983). Somatic embryogenesis: Factors influencing embryogenic response. In P. V. Ammirato (Ed.), *In Vitro Embryogenesis in Plants* (pp. 27–49). Springer.

Bai, B., Su, Y. H., Yuan, J., & Zhang, X. S. (2013). Induction of somatic embryos in *Arabidopsis* requires local YUCCA expression mediated by the down-regulation of ethylene biosynthesis. *Molecular Plant*, *6*(4), 1247–1260. https://doi.org/10.1093/mp/sst065

Bairu, M. W., & Van Staden, J. (2013). Auxin-induced callus formation and somatic embryogenesis in plant tissue culture. *Biotechnology Advances*, *31*(7), 1204–1214. https://doi.org/10.1016/j.biotechadv.2013.04.004

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils – A review. *Food and Chemical Toxicology*, *46*(2), 446–475. https://doi.org/10.1016/j.fct.2007.09.106

Balandrin, M. F., Klocke, J. A., Wurtele, E. S., & Bollinger, W. H. (1985). Natural plant chemicals: Sources of industrial and medicinal materials. *Science, 228*(4706), 1154–1160.

Benderradji, L. (2022). *Avantages et limites de la culture in vitro chez le palmier dattier (Phoenix dactylifera L.)* [Master's thesis, Université Kasdi Merbah Ouargla].

Benmahioul, B. (n.d.). *Culture in vitro* [Course material]. Université Abou Bekr Belkaïd Tlemcen. Retrieved June 7, 2025, from https://elearn.univ-tlemcen.dz/course/view.php?id=1569Elearn

Bhojwani, S. S., & Dantu, P. K. (2013). *Plant Tissue Culture*

Bhat, J. A., & Qadri, R. A. (2018). *In vitro techniques for plant propagation and genetic improvement*. Springer.

Biology Reader. (n.d.). *Meristem Culture – Definition, Protocol, Advantages, and Applications*. Retrieved June 7, 2025, from https://biologyreader.com/meristem-culture.html

Bhat, S. S., & Bhandari, D. M. (2008). *Advances in the conservation of medicinal plants*. Springer.

Bhojwani, S. S., & Razdan, M. K. (1996). *Plant tissue culture: Theory and practice*. Elsevier.

Bone, K., & Mills, S. (2013). *Principles and practice of phytotherapy: Modern herbal medicine* (2nd ed.). Churchill Livingstone Elsevier.

Bousbih, M., Lamhamedi, M. S., Abassi, M., Khasa, D. P., & Béjaoui, Z. (2023). Potential use of two forest species (*Salix alba* and *Casuarina glauca*) in the rhizofiltration of heavy-metal-contaminated industrial wastewater. *Forests*, *14*(3), 654. https://doi.org/10.3390/f14030654

Camper, N. D., Coker, P. S., Wedge, D. E., & Keese, R. J. (1997). In vitro culture of *Ginkgo*. *In Vitro Cellular & Developmental Biology – Plant, 33*(2), 125–127. https://doi.org/10.1007/s11627-997-0019-4

Careers360. (n.d.). Difference between organogenesis and somatic embryogenesis. Retrieved June 7, 2025, from https://www.careers360.com

Cassells, A. C., & Curry, R. F. (2001). Oxidative stress and physiological, epigenetic and genetic variability in plant tissue culture: Implications for micropropagators and genetic engineers. *Plant Cell, Tissue and Organ Culture, 64*(2–3), 145–157.

CGIAR Genebank Initiative. (n.d.). *In vitro | Conservation Module | CGIAR genebanks*. Retrieved April 6, 2025, from https://www.genebanks.org/resources/genebank-operations/conservation/in-vitro/

Chaib, G. (n.d.). *Biotechnology course, 3rd year undergraduate – Plant biology and physiology (BPV)*. University of Frères Mentouri – Constantine 1.

Chevallier, A. (2016). *Encyclopedia of herbal medicine: 550 herbs and remedies for common ailments* (Updated Ed.). DK Publishing.

Davey, M. R., & Anthony, P. (2010). Plant protoplasts: Isolation, culture and regeneration. *Plant Cell, Tissue and Organ Culture, 17*(1), 59–66. https://doi.org/10.1007/BF00043009

Debnath, S. C., Da Silva, J. A. T., & Saxena, P. K. (2017). In vitro culture of medicinal plants: A review. In *Biotechnology for medicinal plants* (pp. 25–57). Springer.

Demarly, Y., & Sibi, M. (1996). *Culture in vitro et amélioration des plantes*. INRA Éditions.

Djennane, S., & Klifatti, A. (1996). Techniques de culture in vitro: Protoplastes et haploïdies chez les plantes supérieures. *Revue des BioSciences, 3*(2), 88–95.

Dossoukpevi, R., Ahanhanzo, C., Gbaguidi, F., Agbangla, C., Agbidinoukoun, A., & Cacaï, G. (2016). Incidence des plantes régénérées in vitro sur les huiles essentielles de deux espèces de *Ocimum* cultivées au Bénin. *Journal of Applied Biosciences*, *99*, 9441–9449.

Dulai, S. L., & Jain, S. M. (2009). Gynogenesis as a tool for haploid production in plants. In S. M. Jain (Ed.), *Haploid production in crop plants* (pp. 295–307). Springer.

Farrelly Mitchell. (2025). *Advantages and disadvantages of micropropagation*. https://www.farrellymitchell.com

Fay, M. F. (1994). In what situations is in vitro culture appropriate to plant conservation? *Biodiversity & Conservation*, *3*, 176–183. https://doi.org/10.1007/BF00055969

Fehér, A. (2019). Callus, dedifferentiation, totipotency, somatic embryogenesis: What these terms mean in the era of molecular plant biology? *Plants, 8*(9), Article 339. https://doi.org/10.3390/plants8090339

Ferreira, M. D. S., Moraes, T. S., & Alves, E. U. (2023). The role of somaclonal variation in plant genetic improvement: A systematic review. *Agronomy, 13*(3), 730. https://doi.org/10.3390/agronomy13030730

Fraga, C. G., Croft, K. D., Kennedy, D. O., & Tomás-Barberán, F. A. (2019). The effects of polyphenols and other bioactives on human health. *Food & Function, 10*(2), 514–528. https://doi.org/10.1039/C8FO01997

Bibliographic references

Gamborg, O. L., Miller, R. A., & Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research, 50*(1), 151–158. https://doi.org/10.1016/0014-4827(68)90403-5

Gao, F., Peng, C., Wang, H., Shen, H., & Yang, L. (2020). Selection of culture conditions for callus induction and proliferation by somatic embryogenesis of *Pinus koraiensis*. *Plant Cell, Tissue and Organ Culture, 143*(2), 397–406. https://doi.org/10.1007/s11240-020-01824-0

George, E. F., Hall, M. A., & De Klerk, G.-J. (2008). *Plant propagation by tissue culture: Volume 1. The background* (3rd ed.). Springer.

GeeksforGeeks. (2024). *Micropropagation*. Retrieved from https://www.geeksforgeeks.org/micropropagation/

Giri, C. C., & Zaheer, M. (2016). Chemical elicitors versus physical stress: Strategies to enhance secondary metabolite production in medicinal plants using tissue culture. *Plant Cell, Tissue and Organ Culture, 126*(1), 1–18.

Grzelak, M., Pacholczak, A., & Nowakowska, K. (2024). Challenges and insights in the acclimatization step of micropropagated woody plants. *Plant Cell, Tissue and Organ Culture (PCTOC), 159*(72).

Guo, B., & Zhao, X. (2013). Haplo-diploidization techniques in plant breeding: Advances and applications. *Plant Cell, Tissue and Organ Culture, 113*(1), 1–10. https://doi.org/10.1007/s11240-012-0221-x

Gurib-Fakim, A. (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular*

Goyal, S., Ramawat, K. G., & Merillon, J.-M. (2011). Biotechnology: Secondary Metabolites. In: *Plant Defence: Biological Control* (pp. 305–319)

Hailu, T., Abera, B., & Gabra Mariam, E. (2013). In vitro mass propagation of *Artemisia* (*Artemisia annua* L.) cv: Anamed. *Plant Tissue Culture and Biotechnology, 23*(2), 165–176. https://doi.org/10.3329/ptcb.v23i2.17518

Heinrich, M., Barnes, J., Gibbons, S., & Williamson, E. M. (2012). *Fundamentals of pharmacognosy and phytotherapy* (2nd ed.). Elsevier Health Sciences.

Haberlandt, G. (1902). Culturversuche mit isolierten Pflanzenzellen. *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften in Wien, Mathematisch-Naturwissenschaftliche Klasse, 111*, 69–92.

Iac.nc. (2023). *L'adaptation des agrumes de Nouvelle-Calédonie à la sécheresse : l'apport de la culture in vitro*. https://www.iac.nc

Iliev, I. A. (2017). Factors affecting the axillary and adventitious shoot formation in woody plants *in vitro*. *Acta Horticulturae, 1155*, 15–22. https://doi.org/10.17660/ActaHortic.2017.1155.2

Jain, S. M. (2010). Somatic embryogenesis in woody plants. In S. M. Jain (Ed.), *Somatic embryogenesis in woody plants* (pp. 1–25). Springer.

Jia, L., Wang, X., & Zhao, M. (2008). The somatic embryogenesis of *Saussurea involucrata* Kar. Et Kir. *Chinese Bulletin of Botany, 25*(1), 85–88.

Kao, K. N., & Tseng, T. S. (1979). Embryo culture and plant development. *Annual Review of Plant Physiology, 30*(1), 333–352. https://doi.org/10.1146/annurev.pp.30.060179.001533

Bibliographic references

Khan, M. I., & Malik, S. A. (2015). *Cryopreservation of plant species: A modern approach to conservation*. Academic Press.

Kumar, V., Mathur, M., & Saxena, S. (2009). Acclimatization of tissue cultured plantlets: From laboratory to land. *Biotechnology Advances*, *27*(6), 711–717. https://doi.org/10.1016/j.biotechadv.2009.06.004

Kozai, T., & Fujiwara, K. (2016). *Micropropagation: Technology and application*. Springer.

Kumar, A., & Reddy, M. P. (2024). Micropropagation: A novel technique for propagation of horticulture crops. *Just Agriculture*, *4*(10), 249–252.

Kolewe, M. E., Gaurav, V., & Roberts, S. C. (2008). Phytochemical production in plant cell culture. *(Note: compléter les détails de la revue ou ouvrage si disponibles)*

Olah, R., Turcsan, M., Olah, K., Farkas, E., Deak, T., Jahnke, G., & Sardy, D. A. N. (2022). Somatic embryogenesis: A tool for fast and reliable virus and viroid elimination for grapevine and other plant species. *Horticulturae*, *8*(6), 508. https://doi.org/10.3390/horticulturae8060508

Lee, J.-S., Kim, C., Kim, I. S., Lee, E. J., & Choi, H. K. (2006). In vitro regeneration of *Phragmites australis* through embryogenic cultures. *Plant Biotechnology*, *8*(1), 21–25.

Loyola-Vargas, V. M., & Ochoa-Alejo, N. (Eds.). (2016). *Somatic embryogenesis: Fundamental aspects and applications*. Springer.

Loyola-Vargas, V. M., & Ochoa-Alejo, N. (Eds.). (2016). *Plant cell culture protocols* (Methods in Molecular Biology, Vol. 1359). Humana Press. https://doi.org/10.1007/978-1-4939-3112-6

Lloyd, G., & McCown, B. (1980). Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by shoot tip culture. *International Plant Propagators' Society Proceedings*, *30*, 421–427

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *The American Journal of Clinical Nutrition*, 79(5), 727–747.

Marchant, M. J., Molina, P., Montecinos, M., Guzmán, L., Balada, C., Fassio, C., & Castro, M. (2021). In vitro propagation of Easter Island *Curcuma longa* from rhizome explants using temporary immersion system. *Agronomy*, 11(11), 2121. https://doi.org/10.3390/agronomy11112121

Manjudevi, M., Thirugnanasampandan, R., Vishnupriya, B., & Gogul Ramnath, M. (2022). Standardization of medium for shoot and callus induction in *Ocimum* species and evaluation of antioxidant and anti-inflammatory activities of eugenol and camphor. *South African Journal of Botany*. [Elsevier B.V. on behalf of SAAB]. Available online 17 February 2022.

Mishra, A. K., Saini, R., & Tiwari, K. N. (2022). Double haploid production and its applications in crop improvement. In *Agricultural Biotechnology: Latest Research and Trends* (pp. 75–101).

Mohamed S. Abbas, Hussein S. Taha, Usama I. Aly, Hattem M. El-Shabrawi, & El-Sayed I. Gaber. (2011). In vitro propagation of ginger (*Zingiber officinale* Rosco). *Journal of Genetic Engineering and Biotechnology*. Received 13 September 2011; Revised 17 November 2011; Accepted 20 November 2011; Available online 16 December 2011.

Murthy, H. N., Lee, E. J., & Paek, K. Y. (2014). Production of secondary metabolites from cell and organ cultures: Strategies and approaches for biomass improvement and metabolite accumulation. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 118(1), 1–16. https://doi.org/10.1007/s11240-014-0467-7

Norrel, H. A. (1973). Totipotency and cell differentiation in plant cells. Plant Physiology Journal, 42(4), 321–328.

Nehnevajova, E., Herzig, R., Erismann, K. H., et al. (2007). In vitro breeding of Brassica juncea L. to enhance metal accumulation and extraction properties. Plant Cell Reports, 26, 429–437. https://doi.org/10.1007/s00299-006-0264-9

Nguyen, T. N., Trung, K. H., & Khanh, T. D. (2024). Current advances in tissue culture of Vietnamese ginseng (Panax vietnamensis Ha et Grushv). Natural Product Communications, 19(8), 1–12. https://doi.org/10.1177/1934578X241279712

New Caledonian Agronomic Institute (IAC). (2023). Research on drought tolerance in Oxanthera spp. Retrieved from https://iac.nc

Preece, J. E., & Janick, J. (Eds.). (1994). Methods in plant propagation. John Wiley & Sons.

Parsamanesh, Z., Bayat, F., & Hedayat, M. (2018). Optimizing in vitro regeneration of Hypericum (Hypericum perforatum L.). Plant Production and Development. Advance online publication. https://doi.org/10.22055/ppd.2018.13440

Pardo, J. E., et al. (2013). Phytoremediation of heavy metals: A promising approach for environmental cleanup. Environmental Science and Pollution Research.

Pan, S. Y., Zhou, S. F., Gao, S. H., Yu, Z. L., Zhang, S. F., Tang, M. K., ,. & Ko, K. M. (2013). New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. Evidence-Based Complementary and Alternative Medicine, 2013, 627375. https://doi.org/10.1155/2013/627375

Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity, 2(5), 270–278.

Pierik, R. L. M. (1997). In vitro culture of higher plants.

Pokhrel, B., & Thapa, B. (2010). In vitro propagation of Rauvolfia serpentina through different explants. Scientific World, 8(8), 76–79.

Ramachandra Rao, S., & Ravishankar, G. A. (2002). Plant cell cultures: Chemical factories of secondary metabolites. Biotechnology Advances, 20(2), 101–153. https://doi.org/10.1016/S0734-9750(02)00007-1

Ramakrishnan, M., Zhou, M., Ceasar, S. A., Ali, D. J., Maharajan, T., Vinod, K. K., Sharma, A., Ahmad, Z., & Wei, Q. (2023). Epigenetic modifications and miRNAs determine the transition of somatic cells into somatic embryos. Plant Cell Reports, 42, 1845–1873. https://doi.org/10.1007/s00299-023-02970-9

Ramawat, K. G., & Mérillon, J.-M. (Eds.). (2008). *Bioactive Molecules and Medicinal Plants*

Rates, S. M. K. (2001). Plants as source of drugs. Toxicon, 39(5), 603–613. https://doi.org/10.1016/S0041-0101(00)00154-9

Ramesh, P., & Suresh, K. (2011). Industrial applications of somatic embryogenesis for secondary metabolite production. In Vitro Cellular & Developmental Biology – Plant, 47(4), 381–392. https://doi.org/10.1007/s11627-011-9385-0

Ramesh, S., Karthikeyan, K., Muruganantham, P., & Chandran, C. (2022). In vitro regeneration of Withania somnifera L. Dunal: A rare medicinal plant. Current Research in Interdisciplinary Studies, 1(3), 1–8.

Rascio, N., et al. (2010). Heavy metal hyperaccumulating plants: How and why do they do it? Journal of Botany. [Volume and pages not provided]

Radiant Psyche. (2025). Healing: 40 Medicinal Plants and Their Uses. Retrieved from https://www.radiantpsyche.com/healing-40-medicinal-plants-and-their-uses

Russo, E. B. (2011). Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. British Journal of Pharmacology, 163(7), 1344–1364.

Saadi, S., & Hamdani, A. (2007). Introduction à la culture in vitro: Techniques de base et applications. Université Ferhat Abbas Sétif.

Saini, H. S., & Sharma, M. (2008). Androgenesis in plants: A review of its potential for the development of homozygous lines. Journal of Plant Biotechnology, 5(3), 47–56.

Sakhanokho, H. F., & Kelley, K. (2009). Cytokinins in plant tissue culture. Methods in Molecular Biology.

Salt, D. E., et al. (1998). Phytoremediation: A novel strategy for the removal of toxic metals from the environment. Trends in Biotechnology

Siddiqui, M. H., et al. (2015). In vitro phytoremediation techniques and their applications. Environmental Science and Pollution Research. [Volume and pages not provided]

Sharma, P., & Kumar, V. (2010). In vitro conservation of medicinal plants: Techniques and applications. Wiley-Blackwell.

Skoog, F., & Miller, C. O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symposia of the Society for Experimental Biology, 11, 118–130.

Smith, R. H. (2012). Plant tissue culture: Techniques and experiments (3rd ed.). Academic Press.

Steward, F. C., Mapes, M. O., & Mears, K. (1967). Growth and organized development of cultured cells. II. Organization in cultures grown from freely suspended cells. American Journal of Botany, 54(8), 755–764. https://doi.org/10.2307/2440117

Sugisawa, H., & Ohnishi, Y. (1976). Isolation and identification of monoterpenes from cultured cells of Perilla plant. Agricultural and Biological Chemistry, 40(1), 231–232.

Sutini, W., Widiwurjani, Purwanto, D. A., & Muslihatin, W. (2018). Polyphenon Extraction Process From In vitro Culture of Camellia sinensis L Callus With Ethyl Alcohol. In Atlantis Highlights in Engineering (AHE), Volume 1, Proceedings of the International Conference on Science and Technology (ICST 2018). Atlantis Press. https://doi.org/10.2991/icst-18.2018.109

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). Plant physiology and development (6th ed.). Sinauer Associates.

Takeda, R., & Katoh, K. (1981). Growth and sesquiterpenoid production by Calypogeia granulata Inoue cells in suspension culture. Planta, 151(6), 525–530.

Thorpe, T. A. (Ed.). (2007). Plant cell culture: Essential methods. Wiley-Blackwell.

Bibliographic references

Yadav, S. K. (2010). Heavy metal toxicity and tolerance in plants. Biological Trace Element Research, 12(1), 25–37.

Violon, C., Sonck, W., & Vercruysse, A. (1984). Comparative study of the essential oils of in vivo and in vitro grown Valeriana officinalis L. and Centranthus macrosiphon Boiss. by coupled gas chromatography—mass spectrometry. Journal of Chromatography A, 288, 474–478.

Vidalis, A., Katinakis, P., & Economou, A. (1985). Effect of genotype and explant age on shoot regeneration in vitro. Plant Cell, Tissue and Organ Culture, 4(2), 145–153. https://doi.org/10.1007/BF00040455

Vidalis, A., Katinakis, P., Economou, A., & Diamantoglou, S. (1989). Physiological responses of explants during in vitro culture. Annals of Botany, 63(1), 1–10. https://doi.org/10.1093/oxfordjournals.aob.a087927

Wang, Q., et al. (2018). In vitro thermotherapy-based methods for plant virus eradication. Plant Methods, 14, Article 1–15. https://doi.org/10.1186/s13007-018-0277-7

Wink, M. (2015). Modes of action of herbal medicines and plant secondary metabolites. Medicines, 2(3), 251–286. https://doi.org/10.3390/medicines2030251

World Health Organization. (2013). WHO traditional medicine strategy: 2014–2023. World Health Organization. https://www.who.int/publications/i/item/9789241506090

World Health Organization (WHO). (2002). Traditional medicine strategy 2002–2005. WHO.

Wang, X., & Jin, Y. (2013). Somatic embryogenesis for cloning elite plants in forestry. Tree Physiology, 33(4), 223–234. https://doi.org/10.1093/treephys/tps127

Webb, D. T., Flinn, B. S., & Webb, M. C. (1989). Developmental pathways in plant tissue culture. Journal of Experimental Botany, 40(1), 99–105. https://doi.org/10.1093/jxb/40.1.99

Wheeler, D. L., Thomas, J. C., & Chandler, J. M. (1985). Morphogenic response of tissues to in vitro stimuli. Plant Science Letters, 38(2), 113–120. https://doi.org/10.1016/0304-4211(85)90038-6

Wang, Q., Cuellar, W. J., Rajamäki, M.-L., Hirata, Y., & Valkonen, J. P. T. (2018). Plant meristem culture: A reliable technique for virus-free plant production. Physiological and Molecular Plant Pathology, 103, 51–57.

Wang, Q., Cuellar, W. J., Rajamäki, M. L., Hirata, Y., & Valkonen, J. P. T. (2008). Plant recovery by shoot tip culture and thermotherapy from plants infected with viruses. In G. Loebenstein & N. I. Katis (Eds.), Control of Plant Virus Diseases (pp. 235–250).

Yadav, S. K. (2010). Heavy metal toxicity and tolerance in plants. Biological Trace Element Research, 12(1), 25–37.

Xavier, J. R., Ramaswamy Gnanam, M., Murugan, M. P., & Pappachan, A. (2012). Clonal propagation of Phyllanthus amarus: A hepatoprotector. International Journal of Biotechnology, 10(1), 1–12.

Zayova, E., Stancheva, I., Geneva, M., Petrova, M., & Vasilevska-Ivanova, R. (2012). Morphological evaluation and antioxidant activity of in vitro- and in vivo-derived Echinacea purpurea plants. Open Life Sciences, 7(4), 698–707. https://doi.org/10.2478/s11535-012