Chapter 1

Introduction

Plants play a significant role in human lives. There wouldn't be any life left on Earth without plants. Approximately three to four lakh plant species have been discovered, and the number is continually growing. They supply us with a wide range of necessities for our everyday needs, including as wood, medicine, shelter, food, air to breathe, clothing to cover our bodies, and other goods that are beneficial to us. Since plants are the planet's principal producers, all other living things rely on them. Human existence on Earth depends heavily on plants, and people have evolved a number of ways to efficiently utilize plant resources. We have seen a significant transformation in life science after the 20^{th} century. Investigations that have been gathered via biotechnology, particularly in the field of agriculture. Genetic engineering is one of the most important uses of biotechnology in plant resource use. Through genetic engineering, scientists may alter a plant's genetic composition to achieve desired characteristics like higher production or resistance to pests and diseases. Thanks to science, genetically modified crops are becoming more and more abundant and nutritious, which is helping to fulfill the world's expanding food need. Another major application of biotechnology is plant tissue culture is Micropropagation, the method for mass production of genetically identical plants from a single parent plant via tissue culture, is called somaclones. Using of disease-rode plants as explants, the approach offers an advantage over previous techniques in that disease-free plants may be developed from diseased plants.

1.1 Introduction to micropropagation

Plants have the ability to regenerate whole plants from individual cells or from groups of cells within a tissue or organ, expressing the entire plant genome, which is opposed to most animal cells this is called totipotency. Plant regeneration is often achieved by the use of explants, which are small amounts of tissue, organs, or isolated callus that are removed from the parent plant and cultured *in vitro* on a specific media. The term "micropropagation" comes from the Greek "micro," which means small, and the English word "propagation," which means to multiply propagules. The

fundamental elements of plant regeneration *in vitro* are utilized by all micropropagation approaches. Micropropagation uses plant cells totipotency to regenerate new plants from small explants. This process involves induced cell division, callus tissue formation, callus proliferation, and multistep differentiation and regeneration, resulting in organogenesis (shoots and/or roots) or somatic embryogenesis (Loberant and Altman, 2010). In a short amount of time, micropropagation will allow the large-scale, multiple production of the same genotype (Cardoso *et al*., 2018). Compared to more conventional methods of propagation such as air layering, grafting, cutting, and seed, commercial production of plants by micropropagation techniques offers various benefits. Rapid propagation techniques have the potential to produce plants free of viruses (Suman, 2017). By using cell and root cultures, the micropropagation technique is used to study plant biosynthesis and the interaction between endophytes and the hostplant. It is also used to maintain the *in vitro* germplasm of elite, rare, and endangered plant species and to implement breeding programmes for a variety of crops and encapsulated seeds (Moraes *et al*. 2021).

The aim of micropropagation is to generate a large number of viable plants that are able to exist in their natural surroundings. For each micropropagation method to be successful, a number of meticulously carried out steps must be taken. In contrast to conventional propagation techniques, micropropagation is a multi-stage procedure where each step is essential to reaching the final objective of producing plants in culture. Every technique of micropropagation, with the exception of somatic embryogenesis, involves five unique stages in order to achieve the objective. Every technique has benefits and drawbacks.

Using sterile conditions and controlled conditions, cells and tissues are grown on various artificial medium in micropropagation approaches. Generally, in any tissue culture medium, the components are majorly classified in four groups excluding sucrose (carbon source) and agar (solidifying agent). These groups are: a) Macronutrients (Nitrogen, Potassium, Phosphorus, Magnesium, Sulphur), b) Micronutrients (Manganese, Boron, Zinc, Cobalt, Copper), c) Iron and chelating agents ($FeSO_4$ and EDTA) and d) organic supplements (vitamins and amino acids) (Nirmal *et al*. 2023) mainly adding plant growth regulator to the medium due to

research on the interaction between these two plant hormones and its effects on cellular functions, growth patterns, and developmental pathways, as well as the importance of auxins and cytokinin in plant differentiation (Su *et al*. 2011). The purpose of adding gelling agents to the culture media is to make it more viscous so that a solid surface may be formed above the nutritional medium to support the tissues and organs of the plant. Agar is a favoured gelling agent because of its remarkable gelling qualities, stability, and lack of interaction with plant metabolism. (Madi Waheed Al-Mayahi and Hussian Ali, 2021). During micropropagation, the culture vessels are typically small and tightly sealed, which causes a noticeable drop in CO_2 when light is present. Sugars are used as a carbon and energy source in the tissue culture medium to make up for this deficiency (Zobayed, 2005). Furthermore, it is commonly acknowledged that the medium's sugar content is essential for controlling the morphogenesis and development of plant organs.

Tissue culture plantlets are grown using this approach in culture containers under carefully monitored conditions. The light intensity that the plants are exposed to is between 2000 and 3000 lux, which is around 30-45 μmol m^{-2} s^{-1}. By offering a 16/8-hour light and dark cycle, this is accomplished. The culture room is meticulously maintained at 28±2°C with a relative humidity of between 40% - 50 %. Tightly sealed culture containers are used to limit the undesired gas exchange between the vessels and the surrounding environment, preventing contamination (Joshi *et al*., 2006).

In vitro plantlets produced by micropropagation must be properly hardened and acclimated in order for their survival post transplanting to be effective. During this procedure, the plantlets are progressively moved from the sterile laboratory setting into polybags that are loaded with soilless medium. In order to replicate the circumstances seen in the natural environment, the plantlets are exposed to high light intensity and reduced humidity levels throughout this transition. The purpose of this 2-4 weeks of hardening and acclimatisation is to help the plantlets get acclimated to the demands of the outside world. The plantlets gain the ability to withstand higher light levels and decreased humidity, which makes them more capable of establishing and flourishing outside of the carefully monitored laboratory environment. Therefore, the process of hardening and acclimatisation is essential to guarantee the survival and long-term growth of plantlets that have been micropropagated (Chandra *et al.*, 2010).

Exploring the economic factors of rose production via tissue culture in India.

For over five thousand years, roses have brought joy to people as decorative flora, serving also as medicinal aids and even culinary delights. Revered for their beauty and grace, they have inspired various expressions of human creativity across art forms (Mileva *et al*., 2021). As cultivated crops, roses hold significant economic value, ranking among the top five ornamental plants globally. In many developed nations, revenue generated from ornamental plant cultivation surpasses that from vegetable and fruit sales. (Shahrin *et al*., 2015). Floriculture denotes the practice of cultivating flowers. The floriculture industry involves farming flowers specifically for commercial purposes, aiming to market the flowers and their derivatives for economic gain within a given economy. Various flowers are cultivated within the floriculture industry, including American marigolds, *ageratum*, annual vinca, daylily, black-eyed susan, and Japanese iris. These flowers are marketed in diverse forms such as cut flowers, cut foliage, and bedding plants. Flowers and their derivatives serve numerous purposes for a wide range of consumers. Structures are adorned with flowers to enhance their beauty. They can also symbolize gifts expressing appreciation and affection, such as when presented for birthdays and Valentine's Day. Additionally, flowers are commonly offered as condolences when mourning the loss of loved ones. (Xia *et al*., 2006).

The capital investment needed for floriculture, including funds, land, and logistics, is substantial. This significant investment contributes to the profitability of the floriculture business. In 2017, the combined global imports and exports of floriculture plants and their derivatives amounted to USD 8.2 billion and USD 8.7 billion, respectively. Floriculture products boast the highest profit margins per unit area compared to other agricultural goods. Developed nations, particularly those in Europe and America, are primary consumers of these products, while major producers include the Netherlands, along with several countries across Asia, Africa, and the Americas, such as China, Japan, Malaysia, Kenya, and Ecuador (Xia *et al*., 2006; Lariviere, 2017). Area under cultivation in India, Tamil Nadu, Karnataka, Madhya Pradesh, West Bengal, Chhattisgarh, Andhra Pradesh, Gujarat, Uttar Pradesh, Assam, and Maharashtra have emerged as significant states for floriculture cultivation (Misra and Ghosh, 2016). According to the Agriculture Statistics of 2020 released by the

Government of India, the total area dedicated to flower crops in the year 2018-19 was 303 thousand hectares (Chauhan *et al*., 2024). India ranks second globally in terms of the total area under floriculture, following China. Flower production was estimated at 2910 thousand metric tons, with loose flowers accounting for 2263 thousand metric tons and cut flowers for 647 thousand metric tons. Fresh and dried cut flowers dominate India's floriculture exports (Anumala and Kumar, 2021)

As per a report from APEDA, the total area allocated for flower crops was anticipated to be approximately 34,000 hectares. This encompassed 24,000 hectares dedicated to traditional flowers like marigold, jasmine, aster, rose, chrysanthemum, and tuberose, with the remaining 10,000 hectares allocated for modern flowers such as carnation, rose, gerbera, gladiolus, and anthurium. Among states, Karnataka leads in production, yielding a total of 253.24 thousand tonnes. Kerala takes the lead in floriculture within its zone, boasting approximately 53.26 thousand hectares dedicated to floriculture cultivation (Sowmya, 2024). Other significant flower-growing states include Tamil Nadu and Andhra Pradesh in the South, West Bengal in the East, Maharashtra in the West, and Rajasthan, Delhi, and Haryana in the North (Malviya *et al*., 2022). Indian roses cultivated in Bengaluru and Pune are in high demand in Europe and the United Kingdom. The Indian Society of Floriculture Specialists anticipates that India's flower exports worldwide are valued at around 30 crores. During Valentine’s Day and Christmas, flower exports from India, particularly exquisite Indian roses, reach their peak. Roses are among the most extensively exported flowers from India (Vahoniya *et al*., 2018). The floriculture industry in India is experiencing remarkable success, driven by high demand both domestically and internationally. It is poised for significant growth in the global flower export market. In 1999-2000, total floriculture production stood at 84,342.90 metric tons, with export earnings of 105.16 crore rupees. By 2023, production had risen to 21024.41 metric tons, accompanied by a substantial increase in export earnings to 707.81 crore rupees for the same period (Sowmya, 2024).

To choose rose as an object of research or even to choose it as a model plant the reason to do so is that rose combine some model traits not found in any other woody species. Other reason are as follows, With a small genome size, extensive biodiversity comprising numerous morphologically and physiologically distinct

species and cultivated varieties, absence of a juvenility period, and recurrent flowering genotypes exhibiting a generation time of approximately one year, along with straightforward vegetative propagation for most genotypes, the ease of generating segregating progeny across various ploidy levels, including between different species, and access to multiple regeneration and transformation protocols, coupled with its close phylogenetic relationship to other rosaceous crops with more comprehensive genomic data available (Debener and Linde, 2009). Moreover, they exhibit distinct morphological, physiological, and genetic traits that are not observable in other model species. These include the synthesis and release of numerous volatile compounds in petals, extensive morphological diversity featuring various flower structures, growth patterns, and thorn formations, as well as a distinct form of meiosis not present in other taxa. Moreover, roses are enduring woody perennials (Krussmann, 1981), offering intriguing opportunities to explore inquiries regarding the generation and preservation of genetic diversity within natural populations across both diploid and polyploid species.

Micropropagation industries play a crucial role in providing pathogen-free plant propagules to the agricultural sector. This contributes to increased agricultural productivity and creates employment opportunities, especially in developing countries like India (Patil *et al*., 2021). The commercial plant tissue culture industry was initiated in Cochin, India, in 1987, when A.V. Thomas and Company - Kerala (AVT) established a small-scale production facility. Collaborating with the National Chemical Laboratory Pune, India, AVT aimed to cultivate superior genotypes of cardamom plants. Over time, this technology underwent iterative improvements and expansion through a partnership between AVT and a UK-based firm, focusing on enhancing production efficiency. In 1988, India-American Hybrid Seeds, located in Bangalore, imported a tissue culture unit and a greenhouse facility capable of producing up to 10 million plants annually (Salunkhe *et al.*, 2022). In 2005, a survey conducted collaboratively by the Department of Biotechnology (DBT) and the Small Farmer Agri-Business Consortium (SFAC) unveiled that primary consumer of tissue culture plants (TCP) comprised State Agriculture Departments, private farmers, and Agri Export Zones (AEZ). In India, notable plant varieties propagated through tissue culture methods in 2003 included banana, ginger, sugarcane, large cardamom,

turmeric, vanilla, orchids, aloe, gerbera, anthurium, and stevia (Patil *et al*., 2021). As of July 2023, the Government of India, via the DBT, has formally acknowledged and authorized 78 commercial tissue culture units nationwide under the NCS-TCP program. According to DBT data, this represents approximately a 20% reduction compared to figures from 2020. A detailed list of state-wise recognized companies is provided in Table 1.1.

1.1.1 Limitations of traditional micropropagation methods

Large-scale micropropagation projects encounter particular challenges that impede the transition from laboratory-based protocols to practical technology for producing desired clones (Cardoso *et al.,* 2018; Gulzar *et al.,* 2020; Kumari *et al.,* 2023).

Higher cost of production

The costs associated with micropropagation primarily centre on manual labour, as emphasized by (Lee *et al*., 2019). Manual labour represents a substantial portion of the expenses linked to micropropagation, presenting a significant hurdle for India to fully capitalize on the potential of *in vitro* culture technology. In India, the adoption of *in vitro* culture technology has been hindered by the considerable labour costs in micropropagation, constituting 60-70% of the expenses associated with producing *in vitro* plants (Purohit *et al.,* 2011; Amare and Dugassa, 2022). Conventional micropropagation systems entail labour-intensive activities like excising and transplanting plants and explants, thereby contributing to the elevated labour costs associated with the process (Cardoso *et al.,* 2018; Johnson *et al.,* 2023).

The composition of culture media used for shoot propagation and rooting is pivotal in determining production expenses. Among the different components of the medium, gelling agents such as agar have a substantial influence, representing 70% of the overall budget (Ebile *et al*., 2022). Moreover, the incorporation of sucrose, priced at approximately INR 40/kg, notably adds to the total cost of the culture media (Dhanalakshmi and Stephan, 2014). This underscores the economic importance of sucrose in the composition of the media. However, it's crucial to acknowledge that the inclusion of agar and sucrose in the medium poses a significant challenge in terms of

contamination. These components can create favourable conditions for contaminants, resulting in additional financial setbacks.

One of the most critical considerations in large-scale micropropagation production is the multiplication rate, with a minimum three-fold increase considered acceptable (Purohit *et al*., 2011). A high multiplication rate is crucial for successful large-scale production, as it reduces the need for frequent subculturing, leading to labour cost savings. Moreover, a high rate of shoot multiplication can partially offset losses due to contamination and challenges in rooting, hardening, and acclimatization processes. It serves as a valuable strategy to mitigate setbacks and optimize production efficiency. However, the micropropagation industry has not experienced the expected rapid growth, primarily due to decreased overall multiplication rates. This trend is evident in the production of ornamental pot plants, where the current annual output globally is 40 million plants, despite having an installed capacity of 110 million plants (Patil *et al*., 2021).

Loss due to contamination

In addition to the decrease in multiplication rates over time, contamination presents a significant challenge during the multiplication phase of micropropagation (Cardoso *et al*., 2018). Contamination can be highly damaging, capable of undoing months of work and becoming a nightmare for micropropagation practitioners. Bacteria and fungi are common contaminants in cultures of many plant species, particularly in large-scale commercial operations (Cassells, 2012; Okoroafor, 2022). These microorganisms may originate from the explant itself or infiltrate the laboratory environment as natural or man-made contaminants (Dubey and Babel, 2022). It's important to note that the use of agar and sucrose in the culture medium also contributes significantly to contamination issues, leading to additional economic losses (Kumari *et al*., 2023).

Hyperhydricity

Shoots cultured *in vitro* are exposed to a distinct microenvironment characterized by high levels of sugar and nutrients, low light intensity, sterile conditions, and high humidity. While these conditions foster rapid growth and multiplication, they can also induce various morphological, anatomical, and

physiological abnormalities in plants (Hazarika, 2006). One notable issue encountered during *in vitro* plant culture is hyperhydricity, which significantly impacts commercial production. Hyperhydric shoots, despite continuing to grow and multiply at acceptable rates, often face challenges in rooting. Even if they can be successfully rooted, they struggle to establish themselves in soil (Makunga *et al*., 2006). Consequently, hyperhydricity presents a major challenge in micropropagation as it impedes the successful establishment of plants in subsequent growth stages. Addressing this issue is essential to ensure the efficient production of healthy plants for commercial purposes (Modi *et al*., 2009).

Liquid culture system for better growth of culture

The success of *in vitro* micropropagation of plants relies heavily on the composition of the media (Gonçalves and Romano, 2013). Traditionally, plant tissue culture is conducted on semi-solid media containing agar. However, the use of costly materials significantly increases the overall expenses of plant cultivation. Moreover, the labour-intensive nature of subculturing further compounds the financial burden. An alternative approach involves the complete elimination of gelling agents, opting instead for a liquid medium for plant cultivation. Liquid media offer several advantages in micropropagation, including reduced costs of producing plantlets, accelerated multiplication rates, and facilitation of automation (Dutta Gupta, 2006; Mehrotra *et al*., 2007). Indeed, the adoption of liquid culture systems offers a significantly improved level of consistency in culturing conditions. Renewing the culture medium can be easily accomplished without the need to modify container structures. Feasible sterilization can be achieved through microfiltration, simplifying the subsequent cleaning of culture containers. Unlike cultivating on semi-solid media, the possibility of using much larger containers becomes feasible, thus reducing transfer times (Jyoti Sahu and Ram Kumar Sahu, 2013). Micropropagation from various species has demonstrated superior performance in liquid medium compared to semi-solid medium. For example, *Solanum tuberosum* L. produced a high number of shoots (Karyanti *et al*., 2022), and more somatic embryos were generated in *Arabica coffee* (Aguilar *et al*., 2022). The liquid medium has shown a better growth effect on shoot elongation in *Turnera ulmifolia* L. (Shekhawat *et al*., 2014), and biomass production in *Lycium barbarum* L. (Ruta *et al*., 2020). Nowadays, *in vitro*

propagation using liquid medium is being increasingly adopted as an innovative and cost-effective method across a wide range of plant species (Melaku *et al*., 2016; Mohapatra and Batra, 2017; Stevens, 2018; Daneshvar Royandazagh, 2019; Premkumar *et al*., 2020; Varutharaju *et al*., 2021; Vyas *et al*., 2021; Muhammet Dogan, 2022; Shekhawat *et al*., 2022). (Table 1.2).

To maintain cultures in an upright position within the liquid medium, it is crucial to consistently utilize a support matrix. This matrix significantly promotes robust shoot growth, particularly with enhanced aeration, facilitating efficient nutrient absorption. Additionally, it helps disperse harmful phenolic exudates throughout the medium. The inherent stability of these supports effectively counteracts the adverse effects of shear stress and mechanical damage, commonly associated with aeration and agitation in shake flask cultures (Dutta Gupta and Prasad, 2010). A solid matrix is indispensable for many plant systems, as it enhances multiplication, facilitates proper rooting, and ensures improved anchorage in different types of culture vessels (Shahzad *et al*., 2017). Utilizing support matrices also brings economic advantages by obviating the need for expensive gelling agents, thereby reducing costs. Additionally, it minimizes expenses associated with washing and cleaning (Cardoso *et al*., 2018). Culturing in this manner can reduce the risk of contamination since subculturing involves adding sterile liquid media only (Nirmal *et al*., 2023). Various mechanical supports are currently available, and their effective application across different plant systems has been demonstrated by various researchers. Furthermore, the need to produce high-quality plants at competitive prices highlights the importance of automating *in vitro* culture processes. Progress has been made in automating micropropagation using techniques such as organogenesis or somatic embryogenesis within bioreactors, offering a promising avenue for cost reduction (Neumann *et al*., 2020). Bioreactors are widely recognized as self-contained, aseptic environments that utilize liquid nutrient solutions. Efficient micropropagation of plants in these systems, aimed at increasing plant yield, requires a deeper understanding of plant physiological and biochemical responses to environmental cues in the culture microenvironment. Moreover, precise manipulation of specific physical and chemical factors is crucial for guiding plant morphogenesis in liquid culture setups. Bioreactors are particularly

suitable for large-scale tissue culture, allowing for the production of multiple plantlets within a single batch (Lee *et al*., 2019).

Moreover, when plant tissues are submerged in a liquid medium, they often undergo oxidative stress, resulting in noticeable changes in plant anatomy, physiology, and survival (Pasternak *et al*., 2005). Another strategy involves the use of temporary immersion of shoots in liquid media followed by transfer to semi-solid medium (Lee *et al*., 2019). The temporary immersion system has been successfully tested and proven beneficial in various plant species, including *Capparis spinosa* L. (Gianguzzi *et al*., 2019), Apple (Kim *et al*., 2020), Sugarcane (da Silva *et al*., 2020), *Lycium barbarum* L. (Ruta *et al*., 2020), *Larix* × *eurolepis* Henry (Le *et al*., 2021), *Brassavola nodosa* L. (Vendrame *et al*., 2023), and *Carludovica palmata* (Minchala-Buestán *et al*., 2023). For the commercial method of micropropagation, an efficient and cost-effective approach has been developed for Malang (*Colocasia esculenta* L.) (Arano-Avalos *et al*., 2020). see table 1.3 for more references

Effects of polyamines in micropropagation

Polyamines (PAs) are water-soluble, low molecular weight, polycationic, aliphatic nitrogenous compounds containing more than two amino groups, and are present in all living organisms. They can exist freely or be associated with other molecules, such as phenolic acids and macromolecules like nucleic acids and proteins (Rakesh *et al*., 2021). In plants, polyamines play a crucial role in regulating various physiological processes, including flower development, embryogenesis, organogenesis, senescence, and fruit maturation. Additionally, they are involved in the plant's response to biotic and abiotic stresses (Chen *et al*., 2019). Recent studies have examined the role of polyamines in plant development and their mechanisms of action through the use of exogenous PAs, PA synthesis inhibitors, and transgenic approaches. Studies has demonstrated that polyamines (PAs) play a significant role in plant growth, stabilizing nucleic acids and membrane structures, enhancing stress resistance, and even supporting plant survival (Sequera-Mutiozabal *et al*., 2016). For instance, studies on *Pinus virginiana* have shown that the individual application of putrescine, spermidine, and spermine can help recover brown tissues into normal callus, achieving a recovery rate of 1.4%, along with increased antioxidant enzyme activity. However, combining these polyamines resulted in lower recovery rates than

when each was applied alone (Tang *et al*., 2004). Polyamines have also been found to enhance the quality of *in vitro* cultures of *Bacopa monnieri*, as they were shown to increase phenolic and flavonoid content, as well as antioxidant activities (Dey *et al*., 2019). In *Citrullus lanatus* (watermelon), putrescine (Put) has shown the most favourable effects on rooting, root number, and root length. In contrast, spermidine (Spd) yielded the best results for the number of responding explants, shoot number, and shoot length. At this concentration of Spd, chlorophyll and carotenoid content were also higher compared to other Spd concentrations and other polyamines (Vasudevan *et al*., 2017). Rajpal and Tomar (2020) described the presence of cadaverine (Cad) in corn coleoptiles, pea, tomato, and *Datura*, demonstrating that cadaverine levels depend on plant age and progressively decrease as the plant ages. This study aims to explore the enhancing effects of polyamines on rose micropropagules under *in vitro* conditions, with the goal of improving their proliferation and multiplication efficiency.

1.1.2 Factors affecting micropropagation

Photoautotrophic micropropagation

Traditional micropropagation methods typically depend on adding sugars to the culture medium as an external energy source for plant growth. This approach can unintentionally lead to less-than-ideal growth and plant health, hindering micropropagation's ability to produce strong, adaptable plants (Afreen, 2005). Several key factors significantly influence plantlet development in micropropagation, including the amount of carbon dioxide and the lighting conditions (Kozai, 1991). Optimizing these factors is essential for the success of micropropagation techniques. In particular, having the right amount of carbon dioxide and appropriate light are crucial for promoting healthy plant growth during micropropagation (Batista *et al*., 2018).

In controlled culture environments, low light intensity and depleted carbon dioxide levels during light exposure can harm the photosynthetic activity of plants. This highlights the importance of maintaining sufficient carbon dioxide and optimal light conditions throughout micropropagation, as shown by Nguyen *et al*. (1999). The negative effects of insufficient light and suboptimal CO_2 concentration on

photosynthesis in these controlled settings emphasize the need for careful management of these parameters to achieve successful plant growth and development during micropropagation. Conventional micropropagation techniques often rely on sucrose supplementation, leading to photomixotrophic growth in plantlets. This can hinder their acclimatization and hardening after tissue culture. A promising alternative is the photoautotrophic approach, pioneered by Kozai (1991). This method minimizes or eliminates sugars from the culture medium, promoting growth aligned with natural photosynthesis. Consequently, it reduces dependence on external energy sources and mitigates photomixotrophy-related challenges. As demonstrated by Soni *et al*. (2021), this approach improves the efficiency and success rates of plantlet micropropagation and establishment *ex vitro*. Furthermore, elevated carbon dioxide levels during cultivation offer significant benefits. Investigation by Mitra *et al*. (1998) suggests enhanced resistance to contamination under elevated CO_2. Additionally, studies by Mohamed *et al*. (2021) and Pinheiro *et al*. (2021) have shown that increased CO_2 promotes stronger root systems, improved plant vigor, and potentially eliminates the need for post-micropropagation acclimatization by enhancing photosynthesis. This streamlining of the acclimatization process simplifies the transition of micropropagated plantlets to *ex vitro* conditions, positively impacting cultivation practices. Study in photoautotrophic micropropagation has revealed a powerful technique for growing plantlets. This method involves cultivating them in a chamber with extra carbon dioxide (CO_2) and light. This enriched environment significantly improves plantlet shoot growth, as evidenced by studies across various plant types. From herbaceous flowering plants like those observed by Joshi *et al*. (2009) and Norikane *et al*. (2010) to woody plants studied by Voelker *et al*. (2016), de Jesus Santana *et al*. (2022), and Luis and Jabín (2023), this approach has shown promise. By providing optimal light and CO_2 conditions, photoautotrophic micropropagation allows scientists and growers to cultivate healthy shoots from tiny plant pieces much more efficiently. This technique has the potential to revolutionize plant propagation for a wide range of species, from flowers and vegetables to trees and shrubs.

Studies on the micropropagation of *Feronia limonia* has identified a promising technique for enhancing shoot growth and multiplication. Studies by Joshi *et al*. (2010) and Aragón *et al*. (2010) demonstrate that enriching the culture environment

with carbon dioxide (CO_2) significantly improves shoot development. This finding suggests a novel and potentially effective strategy for optimizing micropropagation protocols for *Feronia limonia*.

Significantly, it proves advantageous for semi-solid and bioreactor-based liquid cultures. Recent studies underscore its importance across various plant species, such as Plum (Gago *et al*., 2022), vanilla (Luis and Jabín, 2023), and *Salix vamilanis* (Gago *et al*., 2021). Investigations into elevated CO_2 levels in liquid medium bioreactors during shoot growth for *Plum, vanilla,* and *Salix vamilanis* have yielded noteworthy results. This includes considerable growth enhancement and the production of healthy plantlets thereafter (Afreen *et al*., 2002). The presence of higher CO_2 levels in the liquid medium is likely to optimize conditions for plant growth, thereby enhancing photosynthesis and carbohydrate synthesis. Consequently, this positively impacts overall physiological processes, leading to robust development. Expanding the scope of photoautotrophic conditions to include rooting, Hung *et al*. (2016) successfully cultivated rooted blueberry in a liquid medium. Likewise, Vives *et al*. (2017) demonstrated the rooting of stevia through temporary immersions in a liquid medium under photoautotrophic conditions. These examples exemplify how photoautotrophic micropropagation goes beyond mere shoot growth, facilitating successful root establishment (Table 1.4).

Culture vessel environment

Recently, researchers have delved into various aspects concerning the containers utilized for plant cultivation in laboratory settings. These aspects significantly impact the growth of plants. Key container features include the type, internal space availability, size of the opening at the top, whether the top is covered, and the transparency of the container (Huang and Chen, 2005). These features play a crucial role in regulating moisture, light, and heat retention within the container where plants are cultivated, as well as in facilitating air circulation. These factors collectively contribute to optimizing conditions for the vigorous growth and proliferation of plant shoots (Huang and Chen, 2005; Islam *et al*., 2005).

Research has explored the impact of vessel selection, with vented closures proving advantageous across various species. Studies on species such as Hemidesmus

indicus (Manokari *et al*., 2022a), cork oak (Jiménez *et al*., 2011), Carnations (Casanova *et al*., 2008; Majada *et al*., 2000), *Chlorophytum borivilianum* (Joshi and Purohit, 2012), *Mentha* spp. (Islam *et al*., 2005), *Capsicum annuum* (Mohamed and Alsadon, 2011), *Wrightia tomentosa* (Joshi *et al*., 2009), and *Stevia rebaudiana* (Modi *et al*., 2012) have demonstrated an enhanced rate of shoot multiplication and higher fresh weight contents.

Morpho-physiological and biochemical status

Plants cultivated in liquid mediums often exhibit an inclination towards abnormal accumulation of apoplastic water, resulting in anatomical, physiological, and gross morphological irregularities known as hyperhydricity. This anomaly is characterized by leaves with reduced photosynthetic capacity and deficient or absent cuticular wax development (Gaspar, 1991; Isah, 2015). To address this issue in hyperhydrated shoots and roots, various supporting materials have been employed in liquid culture systems (Nirmal *et al*., 2023). Utilizing techniques such as temporary immersion for *in vitro* plant growth (Ramírez-Mosqueda *et al*., 2019; García-Ramírez, 2023) and agitated liquid cultures (Karalija *et al*., 2017; Malik *et al*., 2017) is essential for enhancing micropropagation methods. Understanding these unusual occurrences during different stages of plant growth and comparing them to semi-solid conditions is fundamental before further improving micropropagation techniques.

Morphological and anatomical studies

In order to comprehend the cellular changes occurring during plant regeneration, various parameters have been investigated to observe alterations in cell shapes and structures, typically through electron microscopy (Zafar *et al*., 2019; Jayappa *et al*., 2020). This research has entailed examination of different plant components, including outer skin cells, stomata (tiny openings), the regulating cells of stomata, and the waxy surface layer. The findings have revealed intriguing correlations between stomatal density and proximity, as well as environmental conditions such as light exposure, carbon dioxide levels, ethylene concentration, humidity, and the external growth environment (Vahdati *et al*., 2017; Neto *et al*., 2020; García-Ramírez, 2023).

Recent years have witnessed a significant emphasis on scrutinizing the internal composition of plant leaves cultivated in controlled environments. Leaves developed under such conditions often exhibit underdeveloped middle layers and delicate nutrient transport systems. Consequently, when these plants undergo relocation, they frequently manifest pronounced reactions to the change, commonly referred to as transplantation shock (Isah, 2015; Mani and Shekhawat, 2017). Notably, leaves of plants cultivated in controlled environments typically feature a thinner upper layer with an inadequately developed palisade layer. Additionally, they possess a notable amount of mesophyll air (Isah, 2015). Disparities in leaf structure between naturally grown (*in vivo*) and controlled environment-grown (*in vitro*) plants have been documented (Yanyou *et al*., 2006; Jogam *et al*., 2020). Furthermore, stems of plantlets cultivated in controlled environments tend to be slender and exhibit significantly reduced collenchyma and sclerenchyma supportive tissues compared to those grown in natural outdoor conditions (Pinheiro *et al*., 2021).

Determining the levels of carbonic anhydrase (CA) enzymes in plantlets cultivated in controlled environments can offer valuable insights into their growth rates. Studies investigating the activity of this enzyme in various *in vitro* cultivated plants have revealed a direct correlation between plantlet growth speed and CA enzyme activity (Yanyou *et al*., 2006). Furthermore, these findings suggest that within controlled environments, plantlets with higher carbonic anhydrase activity tend to demonstrate increased net photosynthetic rates and accelerated growth. Such observations serve as a foundation for informed decision-making when selecting an appropriate growth medium for plant tissue culture. The evaluation of carbonic anhydrase enzyme activity as a physiological indicator to assess relative growth rates has been conducted on *Paulownia tomentosa* plants (Lazova *et al*., 2004).

Plantlets raised in controlled environments often struggle with regulating water loss upon removal from their culture containers. This challenge contributes to low survival rates when transitioning these plantlets to conditions outside the controlled environment, referred to as *ex vitro* conditions (Hazarika, 2006). Efforts to refine protocols for successful transplantation have centred on investigating the water status of plantlets. This includes analysing factors such as total fresh weight, dry weight, percentage of water content, and percentage of dry weight under various *in*

vitro conditions. Furthermore, researchers have examined the percentage of water loss at different stages of micropropagation to address wilting issues. These studies aim to establish effective measures for preventing wilting during the transplant process (Joshi *et al*., 2006; Habibi and Purohit, 2019).

Biochemical studies

Plants encounter various challenges stemming from exposure to factors such as oxidative stress, excessive moisture, low temperatures, fluctuating light intensity, and diverse chemicals found in the growth media (Hasanuzzaman *et al*., 2012). When subjected to oxidative stress, plants generate active oxygen species, which can detrimentally impact plant growth by disrupting internal components and metabolic processes. Particularly, if environmental stressors disturb the dark phase of photosynthesis, they trigger the production of a specific active oxygen species called superoxide (O_2) (van Rossum *et al*., 1997). Plants possess a highly efficient enzymatic antioxidant defense system, comprising catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and polyphenol oxidase (PPO) enzymes. This system enables the removal of reactive oxygen species (ROS), thereby safeguarding plant cells from oxidative harm.

In response to stresses such as extreme temperatures, drought, and salinity, various plant species exhibit the accumulation of proline. While its exact role in enhancing plant tolerance to changes in osmotic conditions is subject to debate, proline is thought to contribute to adjusting osmotic balance, neutralizing harmful reactive oxygen species, and preserving the integrity of cell membranes (Caverzan *et al*., 2016). Numerous studies have reported on proline's capacity to enhance plant resistance against abiotic stresses like drought and salinity (Ashraf and Foolad, 2007). Increased levels of proline under stress conditions have been documented in species such as *Ailanthus altissima* (Filippou *et al*., 2014), *Ipomoea batatas* (Fan *et al*., 2012), and *Macrotyloma uniflorum* (Tejavathi *et al*., 2010).

It has been suggested that the antioxidant activity of plants may be attributed to their phenolic compounds. Flavonoids, a class of polyphenolic compounds, are recognized for their abilities to scavenge free radicals, inhibit hydrolytic and oxidative enzymes, and exhibit anti-inflammatory effects (Pourmorad *et al*., 2006).

Studying the levels of soluble sugars provides valuable insights into sugar metabolism and plant absorption dynamics (Rabot *et al*., 2012). Elevated sucrose concentrations in the culture medium lead to significant accumulation of soluble sugars in plantlets. This phenomenon arises because *in vitro* plantlets rely more on nutrients from the culture medium than those generated through photosynthesis (Wilson *et al*., 2001). The buildup of sugars further reduces the osmotic potentials within plants (Kaur *et al*., 2021). Investigations into soluble sugar levels at various micropropagation stages have been undertaken in Lily (Wu *et al*., 2021), revealing an increase in overall carbohydrate content from *in vitro* shoots to tissue culture plantlets in natural conditions. Similar findings have been reported in *Physocarpus opulifolius* L. maxim. (Jagiełło-Kubiec *et al*., 2021) and *Lavandula viridis* (Mansinhos *et al*., 2022) regarding carbohydrate levels during the ex-vitro rooting process.

Additionally, plants cultivated *in vitro* demonstrate a restricted photosynthetic capacity, a phenomenon linked to abnormalities in chlorophyll and reduced pigment concentrations (Eckstein *et al*., 2012).

1.2 Problem statement based on literature review

In India, the extensive challenges associated with tissue culture-based propagation of rose plants, especially concerning large-scale micropropagation initiatives, have presented numerous hurdles. Despite offering several advantages, traditional micropropagation methods impose limitations that impede widespread adoption as a conventional agricultural practice in India. Current constraints include labour-intensive procedures, high production costs due to extensive use of culture media components like sucrose, elevated input costs, contamination issues, and occurrences of hyperhydricity, collectively hindering its universal application and precision. These constraints significantly impede tissue culture-based propagation in the rose, impacting both cost-effectiveness and reproducibility.

Due to these constraints, there is a pressing need for a cost-effective and highly productive micropropagation protocol for rose plant production. This advancement is crucial for sustaining the tissue culture industry. Currently, we heavily rely on traditional micropropagation methods and seek further enhancements

to develop high-quality plantlets with increased multiplication rates of healthy specimens at lower costs.

The initial findings from the liquid culture medium experiments indicated notable enhancements in plant growth, affirming the potential for refining the existing protocol to promote better plant development. Moreover, our objective includes investigating alternative gelling agents, culture vessels, and CO_2 enrichment techniques, with the aim of further augmenting plant production and bolstering the health of plantlets under *in vitro* conditions. Analysing morphological and physiological parameters under these treatments will offer insights into how plants react to varied growth conditions *in vitro*, ultimately enhancing their survival rates when transitioned to *ex vitro* conditions. These efforts are directed towards achieving our desired objectives, which are outlined as follows:

1.3 Objectives

1. Establishing *in vitro* shoot cultures of rose on semi-solid (SS) medium.

2. Multiplying shoot cultures in a liquid culture system (LM) utilizing various support materials, under a CO_2 enriched environment, with different gelling agents, different polyamines, different LSE and employing different culture vessels and stoppers to assess morphological and biochemical parameters.

3. Inducing *in vitro* rooting in elongated shoots grown on SS medium as well as LM, and conducting comprehensive studies on all morphological, physiological, and biochemical parameters in rooted shoots.

4. Conducting *in vitro* and *ex vitro* hardening of rooted shoots.

5. Assessing the genetic fidelity in tissue culture-derived plantlets using molecular markers (e.g., RAPD).

Hypotheses

Alternative gelling agents:

- Using cost-effective substitutes for traditional gelling agents in culture mediums can enhance nutrient availability and reduce production costs.

- These gelling agents are anticipated to improve plant growth by optimizing the physical properties of the medium.

Innovative culture vessels:

- Employing culture vessels with improved designs for ventilation and light penetration may provide a better micro-environment for plantlets.
- Enhanced gaseous exchange and light distribution are expected to stimulate growth and morpho-physiological traits.

CO_2 enrichment:

- Adding controlled CO_2 enrichment in the growth chambers could boost photosynthetic efficiency.
- This approach is expected to enhance biomass production and overall health of rose plants during micropropagation.

Medium types:

- Implementing modifications in both semi-solid and liquid culture mediums can expand the scope of optimization for different growth parameters.
- This dual approach is anticipated to be versatile in addressing the specific needs of rose plant micropropagation.

Positive effects on growth and quality:

- These modifications aim to yield a higher quantity of healthy rose plants with improved morpho-physiological traits compared to traditional methods.

Cost efficiency:

- The proposed changes aim to reduce the cost per plant, making large-scale micropropagation economically viable without compromising quality.

Table 1.1 A list of tissue culture industries in India recognized by the Department of Biotechnology (DBT), organized by states

Sr. No.	States	Total	Recognized
1	Andhra Pradesh	3	2
2	Bihar	2	1
3	Chhattisgarh	6	6
4	Gujarat	22	15
5	Haryana	3	2
6	Himachal Pradesh	3	2
7	Jharkhand	1	1
8	Karnataka	12	8
9	Maharashtra	24	20
10	Madhya Pradesh	6	5
11	Orissa	3	2
12	Punjab	4	3
13	Rajasthan	1	1
14	Tamil Nadu	9	4
15	Telangana	6	3
16	Uttar Pradesh	4	1
17	West Bengal	2	2

Source: www.dbtncstco.nic.in

Table 1.2 Utilization of liquid culture systems for the micropropagation of various plant species.

Sr No.	Plants	Results	References
1	*Pogostemon erectus*	Multiplication rate high compare to solid medium.	Muhammet Dogan, 2022
2	*Spathoglottis plicata*	Better shooting and rooting response.	Shekhawat *et al.*, 2022
3	*Orchid*	Cost effective	Deb and Pongener, 2022
4	*Gentiana kurroo Royle*	1.41-fold Higher biomass	Alphonse and Thiagarajan, 2021
5	*Aerva lanata (L.)*	Higher multiplication rate.	Varutharaju *et al.*, 2021
6	*Juncus rigidus*	Best shoot bud induction and high rooting.	Vyas *et al.*, 2021
7	*Steinernema jeffreyense*	Higer mass production and high recovery rate.	Dunn *et al*., 2020
8	*Vaccinium vitis-idaea ssp.*	2-3 time more multiplication rate compares to solid medium.	Arigundam *et al.*, 2020
9	*Scoparia dulcis L*	Better plant growth compares to semi-solid medium.	Premkumar *et al.*, 2020
10	Bamboo plants	2-3-fold shoot generation increase.	Ara *et al.,* 2020
11	*Mentha × piperita L*	2.5-fold shoot production increase.	Vaidya *et al.,* 2019
12	*Anthurium andraeanum Lind*	Rate of Somatic Embryo increase.	Wang *et al.,* 2019
13	*Citrus × latifolia*	High number of shoots recorded.	Bulbarela-Marini *et al.,* 2019
14	*Lilium candidum*	Increase rate of survive in acclimatization.	Daneshvar Royandazagh, 2019
15	*Jatropha Curcas*	Highest number of multiple shoot buds was recorded.	Singh, 2018
16	*Rosa canina*	Shoot multiplication was 1.5-2 times better than in other systems.	Malik *et al.,* 2018

Sr No.	Plants	Results	References
17	*Plectranthus bourneae Gamble*	Successfully hardened and transferred to greenhouse condition with 83% survival.	Thaniarasu *et al.*, 2018
18	*citrus epicotyl*	High-quality shoots were produced from explants.	Niedz and Marutani-Hert, 2018
19	*Juglans nigra L.*	Long-term survival and proliferation of micro shoots was achieved.	Stevens and Pijut, 2018
20	Pineapple	Increased salinity or drought tolerance.	Gómez *et al.,* 2017
21	*Typhonium flagelliforme*	Liquid culture media were more effective for all the growth parameters.	Rezali *et al.*, 2017
22	*Rosa tomentosa*	Beter growth compare to solid medium.	Malik *et al.,* 2017
23	*Quercus robur*	Increase axillary bud proliferation.	Gatti *et al.,* 2017
24	sugarcane	*in vitro* rooting rate increase.	Nápoles Borrero *et al.*, 2017
25	*Bacopa monnieri L*	Increase antioxidant property.	Wangdi and Sarethy, 2016

Table 1.3 A list of various types of Temporary Immersion Systems used in the micropropagation of different plant species.

Sr. No.	Plant	System	Result	References
1	*Eucalyptus globulus*	Immersion system	Increase multiplication rate	González *et al.*, 2011
2	*Tectona grandis L*	TIS	Increase the rooting rate and survive rate in field.	Quiala *et al.*, 2012
3	*Pistacia vera L.*	TIB (RITA)	Significantly increase shoot number and root numbers.	Akdemir *et al.*, 2013
4	*D. lutea* × *purpurea*	TIS	Weight increase	Welander *et al.*, 2014
5	*Echinacea purpurea*	BIT	Shoot number increase and health plant growth	Welander *et al.*, 2014
6	*Rubus idaeus*	TIS, BIT	Increase shoot length and shoot number.	Welander *et al.*, 2014
7	*Eleocharis dulcis*	TIBS	Increase the number of rooting	Gao *et al.*, 2015
8	*Castanea sativa* × *C. crenata*	CIS	Increase photosynthetic rate and increase shoot number	Cuenca *et al.*, 2017
9	*Prunus avium L*	TIS	Significantly increase number of shoot and root growth	Godoy *et al.*, 2017
10	*Olea europaea L.*	Bioreactor	Shoot length and Shoot no. 2 time Increase	Benelli and Carlo, 2018
11	*Bletilla striata*	TIBS	Increase seed germination rate and shoot length increase	Zhang *et al.*, 2018
12	*Salix viminalis*	RITA	Hight shoot multiplication rate	Regueira *et al.*, 2018

Sr. No.	Plant	System	Result	References
13	*Capparis spinosa L.*	TI	Increase of shoot num 3 time	Gianguzzi *et al.*, 2019
14	*Anthurium andreanum* Lind	PI TI	Shoot number increase 2 time. Shoot number increase 8 time	Martínez-Estrada *et al.*, 2019
15	*Anthurium andreanum*	Bioreactor	Significantly increase shoot no.	Ramírez-Mosqueda *et al.*, 2019
16	*Tectona grandis Linn. F.*	TIBS (RITA)	Decrease Hyperhydricity increase shoot length	Aguilar *et al.*, 2019
17	*Musa AAA*	TIS, TIB, MATIS, SETIS	Plant health increase and leaf number and shoot number	Bello-Bello *et al.*, 2019
18	*Bambusa vulgaris*	TIS	Increase stomatal index and shoot length	García-Ramírez *et al.*, 2019
19	*Alnus glutinosa*	TIS, Platform, RITA	increased the proliferation rates	José *et al.*, 2020
20	*Malus domestica*	(CIB TIB	Significantly increase shoot number and root numbers.	Kim *et al.*, 2020
21	*Saccharum officinarum* L.	Bioreactor	Increase Root and shoot num.	da Silva *et al.*, 2020
22	*Colocasia esculenta*	TI, TIB	Shoot number and shoot length 2-time increase	Arano-Avalos *et al.*, 2020
23	*Agave tequilana*	TIS (BioMINT)	Increase leaves number	Monja-Mio *et al.*, 2020)
24	*Musa sp. (*silk AAB)	TIB	Shoot num. 3-time increase	Uma *et al.*, 2021
25	*Larix* × *eurolepis* Henry	TIB	Increase somatic embryo proliferation rate	Le *et al.*, 2021
26	*Agava angustifolia* Haw	TIS	bigger and better quality of plant	Monja-Mio *et al.*, 2021

Sr. No.	Plant	System	Result	References
27	*Colocasia esculenta*	TI, TIB	Shoot number and shoot length 3-time increase	Mancilla-Álvarez *et al.*, 2021
28	*Salix viminalis L*	TIBS (RITA)	Shoot and leaf growth significantly increase	Gago *et al.*, 2021
29	*Castanea sativa* (Mill)	TIB, CIB	Increase shoot length	Gago, Bernal, *et al.*, 2022
30	*Prunus domestica L*	TIBS (RITA)	Significantly increase number of shoot and decrease plant nacrosis	Gago, Sánchez, *et al.*, 2022
31	*Cannabis sativa L*	RITA, Plant form bioreactors	Increase Shoot length in less concentration of sucrose	Rico *et al.*, 2022

Table 1.4 Studies on the use of CO_2 enrichment during the micropropagation of various plant species.

Sr. No.	Plant	Effect	References
1	*Cannabis sativa L.* (cannabis)	Increase *in vitro* rooting, Increase photosynthesis rate	Pepe *et al*., 2022
2	*Lippia dulcis*	High growth rate, increase photosynthesis pigment and increase antioxidant defense response	Rocha *et al*., 2022
3	*Aristotelia chilensis [Mol.] Stuntz*	Increase biomass production	Trentini *et al*., 2021
4	*Pfaffia glomerata*	accumulation of dry mass, in addition to increasing the photosynthetic rate	Louback *et al*., 2021
5	*Wasabia japonica* (Wasabi)	Plant all over growth increase and photosynthesis rate increase	Hoang *et al*., 2019
6	*Alnus glutinosa* (Black alder)	acclimatization percentages, with survival percentages greater than 85% being achieved.	Arencibia *et al*., 2018
7	*Salix viminalis* (basket willow)	Increase leaf number, dry weight increase and rate of acclimatization	Kaur and Minhas, 2016
8	*Hevea brasiliensis* (rubber tree)	Increase acclimatization rate	Tisarum *et al*., 2018
9	*Castanea sativa*×*Castanea crenata* (hybrid chestnut)	High proliferation rates, multiplication, rooting and acclimatization increase	Chaari-Rkhis *et al*., 2015
10	*Protea cynaroides*	Rooting and leaf number increase 2.7% as compare to control plant	Wu and Lin, 2013
11	*Gerbera*	Increase rooting number leaf num and fresh weight and dry weight	Cardoso *et al*., 2013
12	*Cynara scolymus*	Water contain increase, Water use efficiency increase	Pérez-Jiménez *et al*., 2015
13	‘Golden’ papaya	Improve potential photosynthetic rates (Apot) and improved biomass accumulation	Schmildt *et al*., 2015
14	*Pfaffia glomerata*	Higher dry weight	Corrêa *et al*., 2015
15	*Solanum tuberosum*	Significant different observed Shoot length, root length, leaf area and fresh weight	Kaur and Minhas, 2016

Sr. No.	Plant	Effect	References
16	*Grammatophyllum scriptum*	Increase average root length and shoot length	Sasongko *et al*., 2017
17	*Prunus avium*	Increase photosynthetic rates, height, and leaf number	Pérez-Jiménez *et al*., 2017
18	*Lippia alba*	Increase physical parameters with the increased stomatal density and lignin content for all chemotypes	Batista *et al*., 2017
19	*Sorbus commixta Hedl*	promoted axillary shoot length, number of nodes, axillary shoot fresh weight, axillary shoot dry weight, leaf length, leaf width, and total chlorophyl.	Park *et al*., 2018
20	*Cymbidium, Phalaenopsis*	Increase photosynthesis rate and plant total weight increase	Nhut *et al*., 2018
21	*Cannabis sativa L*	A 97.5% rooting rate	Kodym and Leeb, 2019
22	*Camellia sinensis (L.), O. Kuntze*	Increase dry weight and shoot length	Bag *et al*., 2019
23	Strawberry *(Fragaria x Ananassa cv.* Festival*)*	shoots rooted best on the same medium of elongation	Kepenek, 2019
24	*Physalis angulata*	Increase chlorophyll contain and plant growth	Santos *et al*., 2020

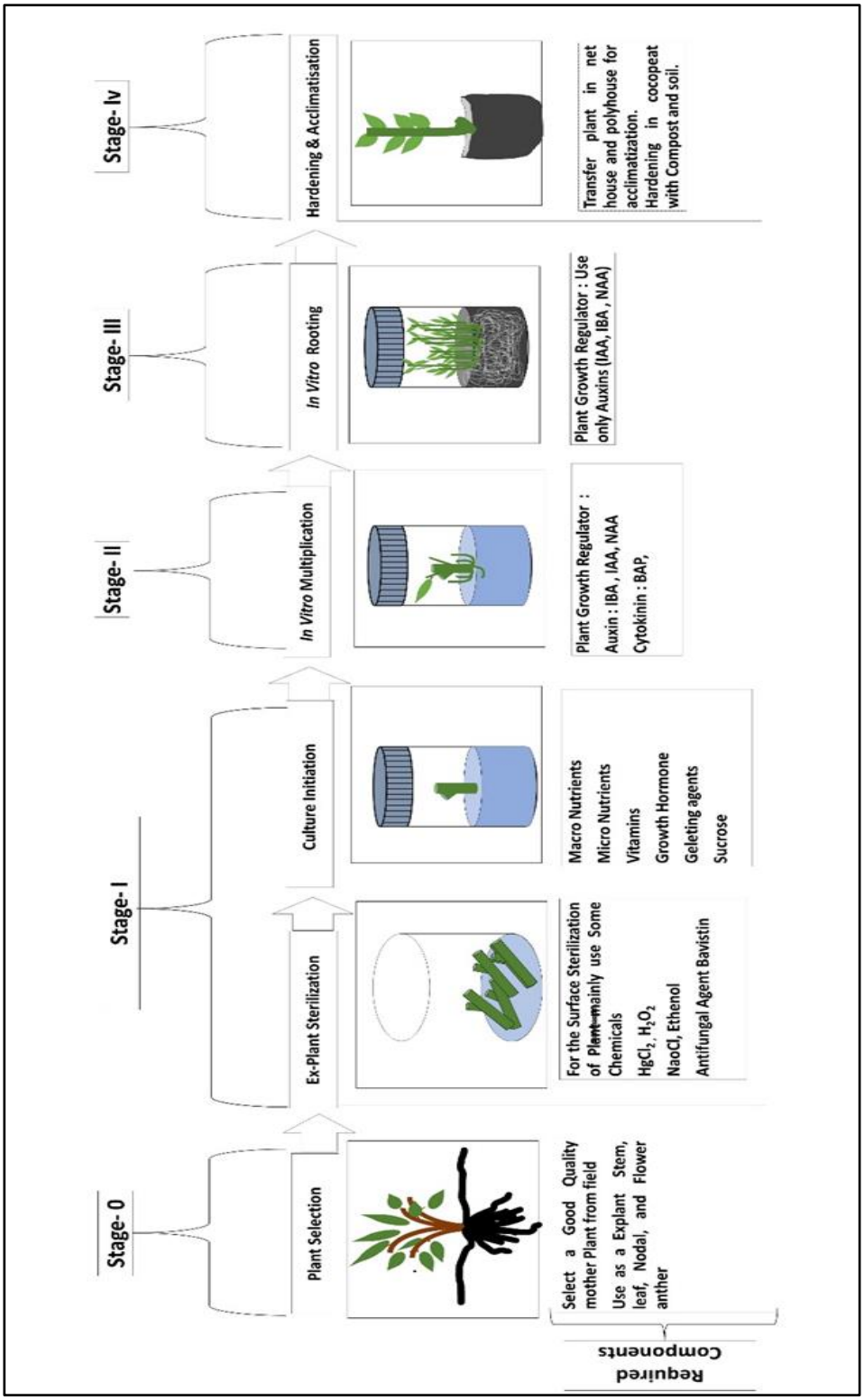

Figure 1.1 Diagram illustrating the various stages of micropropagation (Teraiya *et al*., 2023)