

SYNTHETIC MICROBIAL COMMUNITY: A RHIZOSPHERE MICROBIOME ENGINEERING STRATEGY TO ENHANCE THE SURVIVAL AND RESILIENCE OF TISSUE-CULTURED PLANTS

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ABSTRACT

Micropropagation is a novel plant tissue culture technique used to rapidly produce large numbers of genetically identical plants (clones) under sterile and controlled laboratory conditions. The success of large-scale micropropagation depends on efficient, cheap, and reliable transfer of plants from the lab to the field. However, during the acclimatization stage, tissue cultured plantlets often face challenges such as poorly developed cuticles and stomata, weak roots, and low photosynthetic efficiency, making them sensitive to moisture loss, environmental stress, and microbial competition, resulting in poor survival. This review highlights how beneficial interactions with rhizosphere microbiomes can enhance nutrient acquisition, strengthen stress tolerance, and improve resistance to pathogens, thereby supporting successful establishment of tissue cultured plantlets in soil. It further discusses how Synthetic microbial community (SynComs) strategies applied during acclimatization can significantly improve survival during hardening and increase the capacity of regenerated plants to perform under natural conditions. Although biotization is a well-known strategy in tissue culture, the use of specifically designed SynComs in micropropagation remains largely unexplored. This gap presents a valuable opportunity to improve the scalability and physiological performance of clonally propagated plants. Overall, this review positions SynComs, a tool in rhizosphere microbiome engineering as a promising, sustainable strategy to bridge the gap between *in vitro* propagation and field establishment.

KEYWORDS; SynComs, Rhizosphere Microbiome, Micropropagation, Acclimatization, Biotization

Abbreviations:

SynComs Synthetic microbial community PGPR Plant growth promoting rhizobacteria PGPB

Plant growth promoting bacteria PGPF Plant growth promoting fungi

ARF Auxin response factor

ISR Induced systemic resistance PRR Pattern recognition receptors

EPS Extracellular polymeric substances SA Salicylic acid

ET Ethylene

GAGibberellins

BR Brassinosteroids

JA Jasmonic acid

IAA Indole-3-acetic acid

VOC Volatile organic compounds QS Quorum sensing

ROS Reactive oxygen species AMF Arbuscular mycorrhizal fungi SOD Superoxide Dismutase

CAT Catalase

APX Ascorbate Peroxidase DAPG

2,4-Diacetylphloroglucinol LPS

Lipopolysaccharides

1. INTRODUCTION

Micropropagation is a lab-based method of growing plants in a controlled environment using plant parts like shoot tips, nodes, or small tissue placed on a nutrient medium inside the culture vessels. This method has gained considerable importance in modern agriculture and horticulture because it allows rapid mass production of

healthy, genetically uniform, and disease-free planting material throughout the year. Micropropagation has been successfully adopted for several high-value crops including banana, strawberry, potato, sugarcane, rose, orchid, carnation, gerbera, chrysanthemum, anthurium, date palm, etc. The global micropropagation market reached USD 1.69 billion in 2025 and is projected to grow at a compound annual growth rate (CAGR) of 10.1%, reaching USD 1.86 billion in 2026 and further expanding to USD 2.62 billion by 2030, reflecting the rapidly expanding demand for tissue culture-based plant propagation worldwide (The Business Research Company 2026). Micropropagation also plays a key role in conserving endangered plant species, maintaining genetic integrity, and supporting germplasm preservation programs.

The micropropagation follows a standardized five stage protocol for commercial production. Stage 0 involves selection and preparation of healthy explants under controlled conditions. Stage I includes surface sterilization of aseptic cultures and initial establishment on nutrient media. Stage II focuses on rapid multiplication of shoots using cytokinin rich media. Stage III induces rooting with auxin supplementation. Stage IV comprises of hardening and acclimatization of TC plantlets to *ex vitro* conditions and this stage is widely considered the most critical decision-making phase of the entire tissue culture process, as the survival and successful establishment of plantlets in natural growing conditions ultimately depends on how effectively they adapt during acclimatization stage. During the hardening phase, when plantlets are transferred from sterile environment to greenhouse or field conditions, a high proportion of plants fail to survive. Survival during acclimatization is often limited, with reported success rates varying from 40-80% depending on species and methods (Tesfa and Admassu 2016). This issue limits the practical and commercial use of micropropagation.

The reduced performance of tissue cultured (TC) plantlets during acclimatization is predominantly due to several physiological and structural limitations induced by *in vitro* conditions. As a result, TC plants often show thin cuticles, poorly functioning stomata, weak vascular tissues, and low photosynthetic efficiency. Another important consequence of sterile *in vitro* growth is the lack of interaction with microbial communities. Unlike conventionally propagated plants, micropropagated plantlets do not experience contact with the diverse microorganisms present in the soil. Without these interactions, plantlets have weak immunity and struggle to survive alongside soil microbes when they're first moved to *ex vitro* conditions. In natural systems, plant roots and soil microbes engage in continuous chemical communication that shapes nutrient exchange, stress responses, and immune development. But micropropagated plants are not be ready for these interactions, causing their root zone to be unstable when they are first growing in the soil.

Introducing microbes to *in vitro* grown plantlets, helps them get along with the right microbes before harmful ones take over. Several recent reviews highlight the potential of such plant growth-promoting microbes to enhance micropropagation efficiency, particularly during acclimatization (Guru et al. 2026). Beneficial rhizosphere microorganisms support plant growth and survival through multiple mechanisms. Many Plant growth promoting bacteria (PGPB) produce phytohormones such as auxin, gibberellins, and cytokinins, which stimulate shoot and root development and increase water and nutrient uptake. Some bacteria and fungi solubilize phosphate or fix atmospheric nitrogen, helping plant nutrition when root systems are still developing. Other microbes produce siderophores, antibiotics, and lytic enzymes that limit soil-borne pathogens and improve plant resistance (Kaari et al. 2022). Microbial inoculants improve the resilience and survival of tissue-cultured plants under variable *ex vitro* conditions. Synthetic microbial community (SynCom) represent a logical extension of tissue culture technology by restoring microbial functions absent in aseptically grown plants. This review provides an overview on plant microbe interactions to synthetic consortia design encompassing its application for enhancing survival of tissue cultured plants.

2. Challenges in establishment of tissue culture plants under *ex vitro* conditions

Tissue cultured plants face several challenges when they are transferred from laboratory conditions to the outside environment, and these problems are quite different from those encountered by plants grown from seeds or through conventional propagation methods. As tissue cultured plantlets are raised under controlled, sterile, nutrient-rich laboratory conditions, they often exhibit abnormal morphology, anatomy, and physiology, including thin cuticles, non-functional stomata and low chlorophyll content (Mohammed et al. 2022). When these plantlets are suddenly exposed to *ex vitro* environments characterized by low humidity, high light intensity, fluctuating temperatures, and abundant microbial populations, they encounter high mortality. This occurs because they are unable to regulate water loss efficiently, generate sufficient energy through photosynthesis, or effectively compete with soil microbes (Chandra et al. 2010a; Mohammed et al. 2022). Additional critical challenge faced by tissue-cultured plants is the underdeveloped root system, which typically shows poor root hair formation, limited lateral root development, and inadequate vascular connections (Table 1).

The absence of beneficial microbes increases susceptibility to pathogen attack during the sensitive acclimatization phase. Without established biocontrol organisms and without prior activation of induced systemic resistance (ISR), tissue-cultured plants lack the layered defence mechanisms that are normally present in field-grown plants (Martins et al. 2023a; Dubey et al. 2025). Nutrient uptake is another major obstacle for tissue cultured (TC) plants during their transition from *in vitro* conditions to soil. The readily available nutrients in culture media do not adequately prepare plantlets for real soil environments, where phosphorus is largely

insoluble and nitrogen is often present in limited or fluctuating forms (Jacoby et al. 2017a; Griffin et al. 2024). In addition, some TC plantlets as exposed to excessive levels of phytohormones develop abnormal morphological and anatomical features and are commonly referred to as vitrified or hyperhydricity plants (Griffin et al. 2024). This nutrient related stress becomes particularly severe during the initial weeks after transplanting, when root systems are still immature and not yet capable of effectively supporting beneficial microbial associations (Han and Yoshikuni 2022).

Table 1: Physiological and Anatomical comparison between *In vitro* and Field grown Plants

Table 1

Salient Features	<i>In vitro</i> Plantlets	Field grown Plants	Challenge for Acclimatization	References
Stomata	Often non-functional permanently open circular shape; unable to close in response to darkness or ABA.	Elliptical, functional closure mechanism responsive to environmental stimuli.	Rapid water loss (wilting): Uncontrolled transpiration leads to rapid desiccation upon transfer to lower humidity.	(Sharma et al. 2023)
Cuticle	The cuticle becomes thin or absent because the plant's wax has less oils and protective chemicals (alkanes, wax esters, and triterpenoids), making it thinner and weaker which results in reduced	Thick, fully developed wax layer acting as a transpiration barrier.	Desiccation & Pathogen Entry: Lack of a waxy barrier increases cuticular transpiration and susceptibility to microbial invasion.	(Sharma et al. 2023)

	epicuticular wax deposition.			
Photosynthesis	Low rate; mixotrophic or heterotrophic metabolism dependent on sugar in media; low Rubisco activity.	High rate; fully autotrophic; high Rubisco activity and carbon fixation capacity.	Metabolic Starvation: Plantlets must rapidly switch to autotrophy when sugar is withdrawn, often leading to a temporary energy deficit.	(Sharma et al. 2023)
Root System	Brittle, few or no root hairs; poor vascular connection between root and shoot.	Fibrous or taproot system; abundant root hairs; strong vascular connections.	Poor Water/Nutrient Uptake: Inefficient absorption capabilities which does not match the high transpiration demand.	(Chandra et al. 2010b)
Nutrient uptake	Nutrients are directly available in the growth medium; roots are weak and cannot absorb actively	Roots actively absorb water and nutrients from soil with help of fungal and root hairs	Weak roots fail to absorb enough nutrients from soil causing poor growth and deficiencies and ultimately plant death.	(Hazarika 2006)

Microbiome	Sterile / Aseptic (Microbiologically naive).	Complex "holobiont" with diverse rhizosphere and endosphere communities.	Pathogen Susceptibility: Lack of protective beneficial microbes causes vacant niches for opportunistic pathogens (e.g. <i>Pythium</i> sp.).	(Sharma et al. 2023; Castro Molina et al. 2025)
Excess Phytohormone	Too many growth hormones are added to media, causing abnormal and unhealthy plant growth.	Plant hormones are naturally balanced and produced according to environmental needs and conditions.	Extra hormones remaining in plant tissues disturb normal growth, causing weak roots and poor survival and hyperhydricity	(Pasternak and Steinmacher 2024)

3. Rhizosphere Microbiome: Overview and Functions

The term “rhizosphere” was coined by the German agronomist Lorenz Hiltner in 1904, describing the soil zone immediately surrounding the plant root, filled with microorganisms influenced by chemicals released by roots (Hartmann et al. 2008). Whereas “microbiome” represents the entire community of microorganisms including bacteria, fungi, archaea, viruses, and Protista, that coexist within a specific environment, which influence ecological processes and host physiology. The rhizosphere surrounding plant roots is where active interactions occur among roots, soil, and a dense, active microbiome (Afridi et al. 2022a). The rhizosphere microbiome is a community of microorganisms, including bacteria and fungi, that live on plant roots and helps the plant growth and development. The rhizosphere microbiome includes many types of active microbes, such as plant growth-promoting rhizobacteria (PGPR) like *Pseudomonas fluorescens*, *Herbaspirillum seropedicae*, *Azospirillum brasilense*, *Bacillus sphaericus*, as well as other important PGPR genera including *Azotobacter*, *Rhizobium*, *Burkholderia*, *Enterobacter*, *Serratia*, and *Streptomyces*, alongside fungi such as *Trichoderma viride*, *Piriformospora indica*, and *Arbuscular mycorrhizal* fungi (AMF; e.g. *Glomus spp.*)

Plants release substances from their roots, such as sugars and organic acids, and microbes can sense these chemicals and move toward the roots, where they attach and try to survive. Some plants release certain compounds to attract helpful microbes while using other chemicals to keep harmful ones away. This ongoing interaction changes the metabolism of both sides, increasing the production of important compounds like amino acids, sugars, and protective chemicals (Halder and Sengupta 2015). The rhizosphere microbiome is influenced not only by substances released from roots but also by environmental conditions. Microbes support plant growth by making nutrients like nitrogen, phosphorus, and iron more available, producing plant hormones, siderophores, antibiotics and lytic enzymes (Table 2). These activities improve root growth, increase plant biomass, and enhance tolerance to environmental stresses. Specifically under drought conditions, PGPR mitigate water scarcity by producing exopolysaccharides to enhance soil moisture retention, accumulating compatible solutes like proline and trehalose to maintain cellular turgor, and secreting ACC deaminase to suppress stress-induced ethylene levels that would otherwise inhibit plant development (Dhiman et al. 2026). In nature, plants are constantly interacting with a wide variety of organisms, including bacteria, fungi, and other microbes from all three domains of life (Bacteria, Archaea, and Eukarya). To protect themselves, all plants have an innate immune system, which can detect invading microbes and defend against potential plant pathogens. This immune system not only fight harmful microbes, it also influences which microbes can live in and around the plant, helping shape the plant's microbiome (Hacquard et al. 2017). Basically, it's all about communication between plants and microbes.

Table 2: Beneficial rhizosphere microbes and its role in plant growth and development

Table 2

Microorganism	Type	Key Mechanism	Benefit to TC Plants	Reference
<i>Trichoderma harzianum</i>	PGPF	Mycoparasitism; secretion of cell wall-degrading enzymes (chitinases/glucanases)	Increases drought tolerance and antioxidant activity (SOD, CAT, APX).	(Iqbal 2026)
<i>Pseudomonas fluorescens</i>	PGPR	Siderophore production, ISR induction	Biocontrol, pathogen suppression	(Afridi et al. 2022b)
<i>Azospirillum brasilense</i>	PGPR	N ₂ fixation, IAA production	Root development and lateral roots proliferation	(Nowak 1998a; Guru et al. 2026)
<i>Bacillus subtilis</i>	PGPR	Antibiotic production Phosphorus solubilization	Biocontrol, stress tolerance	(Castro-Restrepo et al. 2022)
<i>Bacillus megaterium</i>	PGPR	High ACC deaminase activity SA production.	Significantly increases stem height and root length in micro-plants improves dry biomass by 70%.	(Castro Molina et al. 2025)
<i>Bacillus velezensis</i> S141	PGPR	Production of phytohormones (auxin and cytokinin).	Increases, dry weight and root-shoot development in soybean	(Thepbandit and Athinuwat 2024; Iqbal 2026)

<i>Rhizobium etli</i>	PGPR	Symbiotic N ₂ fixation, QS mediated stress response	N nutrition, drought survival	(Dundas and Dinneny 2022)
<i>Rhizobium tropici</i> CIA1899	PGPR	IAA and SA biosynthesis; Nitrogen fixation,	Promotes <i>in vitro</i> shoot elongation;	(Castro Molina et al 2025)
		Phytohormone modulation	ensures high survival during acclimatization and increases potential sites for tuberization	
<i>Pseudomonas putida</i>	PGPR	IAA synthesis, root morphology improvement	Lateral root and root hair formation	(Nowak 1998b)
<i>Pseudomonas stutzeri</i> A1501	PGPR	Nitrogen fixation and ammonia oxidation.	Improves shoot/root growth and overall yield in maize	(Thepbandit and Athinuwat 2024)
<i>Trichoderma asperellum</i>	PGPF	Mycoparasitism, enzyme production	Pathogen control, ISR activation	(Castro-Restrepo et al. 2022)
<i>Trichoderma viride</i>	PGPF	Root colonization, VOC production	Root growth, biocontrol	(Afridi et al. 2022b)
<i>Aspergillus niger</i>	PGPF	Phosphate solubilization (mineralizes organic Phosphorus).	Increases uptake of N, P, K, Mg, and Ca in apple	(Thepbandit and Athinuwat 2024)
<i>Piriformospora indica</i>	PGPF	Root endophyte, Phosphorus uptake	Drought tolerance, biomass increase	(Afridi et al 2022b)
<i>Glomus intraradices</i> (AMF)	Fungi	Uptake of soil phosphorus	Increase shoot growth and runner production in strawberry.	(Vestberg 1992)
<i>Glomus</i> spp. (AMF)	Fungi	Phosphorus solubilization, extended root surface	Stress resilience, nutrient access	(Afridi et al 2022b)

<i>Glomus mosseae</i> (AMF)	Fungi	Signaling via underground common mycorrhizal networks (CMNs).	Conveys resistance induced signals to neighboring plants improves nutrient/water access.	(Li et al. 2021 Iqbal 2026)
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<i>Clonostachys rosea</i>	Fungal control (BCA)	Biological agents; Mycoparasitism; enzymatic degradation of pathogens.	Improves post-harvest fruit quality and shelf life in strawberry.	(Iqbal 2026)
<i>Bacillus pumilus</i>	PGPR	Nitrogen fixation and enhanced Nitrogen uptake.	Significantly increases shoot and root fresh/dry weight in tomato plant.	(Thepbandit and Athinuwat 2024)
<i>Janthinobacterium sp</i>	Bacteria	Broad-spectrum antagonism (antibiosis)	Directly inhibits soil-borne pathogens like <i>Pythium ultimum</i> and <i>Rhizoctonia solani</i>	(Li et al. 2021)

<i>Streptomyces sp.</i>	Actinobacteria	Nitrogen fixation, Antioxidant enzyme activation (SOD) Antibiotic production.	Increases flavonoid content and photosynthetic rate acts as host-plant resistance inducer against <i>Botrytis cinerea</i>	(Rana et al. 2025)
<i>Arbuscular Mycorrhiza Fungi (AMF)</i>	Fungi	Production of secondary metabolites (phenols, flavonoids, and carotenoids)	Stimulate mycorrhizal hyphal branching and spore germination	(Rana et al. 2025)
<i>Cyanobacteria (e.g. Anabaena, Spirulina)</i>	Cyanobacteria	Atmospheric N ₂ fixation Synthesis of vitamins, hormones, and enzymes.	Boosts plant immunity against <i>Fusarium wilt</i> increases photosynthetic pigments (carotene) and protein content	(Rana et al. 2025)
<i>Sinorhizobium fredii</i>	PGPR	ACC deaminase activity Phytohormone biosynthesis and Nitrogen fixation	Increases shoot length by 33.2% and root length by 29.6% in micropropagated potato	(Castro Molina et al. 2025)
<i>Lysinibacillus varians KUBM17</i>	PGPR	Nitrogen fixation and Siderophore production.	Supports plant growth (e.g., radish) specifically under cadmium stress conditions.	(Thepbandit and Athinuwat 2024)
<i>Paenibacillus mucilaginosus</i>	PGPR	Potassium solubilization via organic acid production.	Specifically promotes the healthy growth of apple seedlings.	(Thepbandit and Athinuwat 2024)

3.1 Root Colonization and Spatial Dynamics

Root colonization refers to the process by which soil microbes reach plant roots, attach to the surface, proliferate, and persist over time (Saeed et al. 2021; Liu et al. 2024). Microbial distribution along the root is not uniform. Higher microbial densities are typically observed in younger root regions, such as the root tip and sites of lateral root emergence, as these zones exude greater quantities of nutrients and signaling compounds (Galindo-Castañeda et al. 2024). Therefore, just detection of microbes in the rhizosphere does not necessarily indicate true colonization. Effective colonizers are those that remain stably attached and occupy specific root niches over extended periods. Although plant roots release sugars and other small nutrients to attract a wide range of microorganisms, only a limited number of microbes successfully establish themselves (Galindo-Castañeda et al. 2024).

The microbes find their way around the rhizosphere through a process called chemotaxis, where they sense and move toward chemical signals in the soil (Liu et al. 2024). Soil bacteria do this using special receptors on their cell membrane, called methyl accepting chemotaxis proteins (MCPs), which detect substances released by roots like organic acids and sugars and guide the bacteria toward nutrient rich area (Upadhyay et al. 2022; Liu et al. 2024). To actually move toward these areas, bacteria use different methods, such as swimming with flagella, swarming together, or creeping across surface (Liu et al. 2024). They can also use a method called twitching motility, where tiny hair like structures called type IV pili extend and pull back, helping bacteria crawl along the root surface to find the best spot to settle or enter (Hardoim et al. 2008; Liu et al. 2024). Certain microbes aggregate into structured communities that enhance survival under environmental stress and reduce the likelihood of displacement by water flow. These microbial assemblages, often referred to as biofilms, play a critical role in establishing stable root colonization (Rohrbacher and St-Arnaud 2016; Upadhyay et al. 2022). Biofilms consist of microbial cells embedded within extracellular polymeric substances (EPS), supported by adhesion proteins and biofilm specific gene regulatory networks. At the molecular level, biofilm formation involves coordinated production of EPS, activation of adhesion-related proteins, and expression of genes associated with community development (Martins et al. 2023b).

In few cases internalization, happens when microbes like *Corynebacterium flavescentis*, *Serratia spp*, *B. pumilus*, *Azoarcus sp. BH72*, *Burkholderia sp. PsJN* etc. move from the root surface into the plant's internal tissues. This can happen passively, through natural openings such as cracks formed where lateral roots emerge, or through wounds caused by pathogens or predators (Hardoim et al. 2008; Liu et al. 2024). Some microbes take a more active approach, releasing enzymes like cellulases and pectinases that break down parts of the plant's cell walls to create an entry point (Hardoim et al. 2008; Upadhyay et al. 2022). Once inside, these microbes multiply in the spaces between cells in the root's outer layer (Hardoim et al. 2008). A few specialized bacteria go even further, breaking through the root's inner barrier to reach the xylem the plant's internal transport system which allows them to spread upward into stems and leaves (Hardoim et al. 2008; Liu et al. 2024). This phenomenon is particularly evident in tissue culture plants, where studies have revealed that a complex microbiome can survive within tissues previously regarded as sterile, such as the meristematic tissues of micropropagated plants (Hardoim et al. 2008). These plant-microbe associations are so fundamentally integrated into plant biology that establishing transplants in the complete absence of bacteria remains widely recognized as challenging (Hardoim et al. 2008). Moreover, since bacteria can be transmitted through various forms of propagated plant material including cuttings and tissue cultured propagules they are able to colonize subsequent plant generations, thereby contributing to the host's successful establishment even under adverse environmental conditions (Hardoim et al. 2008).

3.2 Molecular communication between microbes and plants

Plant microbe interactions in the rhizosphere are regulated by molecular recognition systems that allow plants to distinguish beneficial microbes from potential pathogens and to mount precisely controlled responses. Plants possess an innate immune security system that detects invading microorganisms through specialized cell surface proteins known as pattern recognition receptors (PRRs). These receptors recognize conserved microbial molecules termed microbe-associated molecular patterns (MAMPs) (Dubey et al. 2025). Recognition of MAMPs activates MAMP triggered immunity (MTI), which constitutes the first layer of plant defense. During beneficial interactions, MTI is initially induced but subsequently attenuated through hormonal crosstalk involving salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) signaling pathways (Dubey et al. 2025). This immune modulation has been extensively characterized in rhizobial symbiosis. In the early stages, rhizobia activate MTI responses; however, they later suppress excessive defense activation by producing ACC deaminase, which reduces ethylene levels, and by modulating SA, JA, and ET signaling networks (Dubey et al. 2025). Such hormonal adjustments enable symbiotic microbes to coexist without triggering strong immune reactions that would otherwise restrict colonization. Understanding these mechanisms is particularly important for designing microbial inoculants capable of efficiently colonizing tissue cultured (TC) plants without inducing detrimental defence responses that impair growth.

The molecular interaction between plant growth-promoting rhizobacteria (PGPR) and plants begins with a complex signal exchange in the rhizosphere (Backer et al. 2018; Pathak et al. 2026). Once microbes reach the root, PGPR utilize extracellular polymeric substances (EPS), adhesins, and fimbriae to attach to the root epidermis, there forming biofilms that facilitate a stable environment for the continuous exchange of metabolites and signaling molecules (Pathak et al. 2026). Direct growth promotion occurs through the microbial synthesis of phytohormones, most notably indole-3-acetic acid (IAA) and gibberellins, which affect root architecture and cell division to increase nutrient and water acquisition (Backer et al. 2018; Salman and Avinash 2025; Pathak et al. 2026). To reduce environmental stress, many PGPR produce the enzyme ACC deaminase, which degrades the ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC), effectively lowering "stress ethylene" levels that would stunt plant development (Backer et al. 2018; Salman and Avinash 2025; Pathak et al. 2026). Additionally, PGPR can trigger Induced Systemic Resistance (ISR), a process that increases the plant's internal defense pathways (such as jasmonic acid and ethylene signaling) to resist future pathogen attacks (Backer et al. 2018; Salman and Avinash 2025; Pathak et al. 2026). A carotenoid derived molecules known as strigolactones (classified as phytohormones) are also released from plant roots via genes like *CCD7*, *CCD8*, and *MAX1* (Boyno et al. 2023). Strigolactones are fundamental for the recognition of plants by arbuscular mycorrhizal fungi, stimulating hyphal branching through fungal responses involving genes such as *AM1*, *AM2*, *AM3*, and *AM11*, while plant genes like *RAM1*, *STR*, *EXO70I*, and *MtENOD11* that support fungal entry and arbuscule development (Park et al. 2015; Boyno et al. 2023). These chemically mediated signaling events ensure targeted recruitment of compatible microbial partners. Under nutrient stress, plants secrete flavonoids and strigolactones in the soil to attract microbes that will support plant growth mechanisms; together these are referred to as molecular communication (Boyno et al. 2023; Dubey et al. 2025). The interaction is genetically controlled by plant symbiosis (Sym) genes and LysM receptor kinases recognizing microbial patterns (Boyno et al. 2023; Dubey et al. 2025). Central regulator in this process is the GRAS family of transcription factor RAM1 which is required for the branching of arbuscules and controls the expression of structural genes such as *EXO70I* and *STR* (Park et al. 2015). Additionally, the expression of RAM1 and with it its interactant RAD1 is regulated by DELLAs, establishing a tight regulatory network of transcription that regulates symbiotic interface development (Park et al. 2015).

3.3 Microbial growth promoters and plant growth

Plant growth-promoting microbial metabolites comprise a core group of bioactive compounds that directly influence plant development and stress adaptation. These include phytohormone-like substances such as indole-3-acetic acid (IAA), cytokinins, and gibberellins; ethylene-modulating factors associated with ACC deaminase activity; and nutrient-mobilizing agents such as siderophores and organic acids (Jeyanthi and Kanimozhi 2018). Among these, auxins-especially indole-3-acetic acid (IAA) is the most extensively studied microbial growth-promoting compounds (Olanrewaju et al. 2017). Numerous rhizosphere bacteria synthesize IAA *via* distinct biochemical pathways, including the indole-3-pyruvate and indole-3-acetamide routes (Li et al. 2018). Microbial-derived IAA enhances primary root elongation, stimulates lateral root initiation, and promotes root hair proliferation, making it particularly advantageous for tissue-cultured plants that typically possess weak or poorly developed root systems (Vacheron et al. 2013).

Cytokinins represent another important class of microbial hormones that promote plant growth by regulating cell division, shoot development, and delaying senescence. Rhizosphere bacteria synthesize cytokinins through the isopentenyl transferase pathway, generating compounds such as zeatin and isopentenyladenosine (García De Salamone et al. 2001). The balance between auxin and cytokinin signaling determines root to shoot ratios and overall plant architecture, a factor that is particularly significant for successful establishment and stress resilience of tissue-cultured plants (Kurepa and Smalle 2022). Additionally, ACC deaminase-producing microbes support plant growth by lowering ethylene concentrations, thereby preventing ethylene induced inhibition of root elongation and stress sensitivity.

Beyond direct growth stimulation, microbes play a important role in stress mitigation by controlling the levels of ethylene, a gaseous stress hormone, at high concentration it inhibits root and stem elongation and triggers premature senescence (Jeyanthi and Kanimozhi 2018). To prevent these growth limiting effect, many plant growth-promoting rhizobacteria (PGPR) produce the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, which degrades the ethylene precursor, ACC, into ammonia and α -ketobutyrate (Vacheron et al. 2013). This enzymatic activity effectively lowers the internal ethylene concentration inside the plant, preventing the inhibition of primary root growth and increasing the plant's overall resilience to environmental stress (Kurepa and Smalle 2022). Furthermore, microbial derived gibberellins, specifically GA3, enhances plant morphology by increasing cell division, stem elongation, and the extension of both primary and secondary roots (Jeyanthi and Kanimozhi 2018). Diverse PGPR strains, including those from the genera *Azospirillum*, *Pseudomonas*, and *Bacillus*, have been documented to synthesize physiologically active gibberellins that directly correlate with increased plant biomass and the regulation of root hair abundance (García De Salamone et al. 2001).

3.4 Microbial metabolites and plant defence

Defense related secondary metabolites are small, structurally diverse compounds produced by both plants and rhizosphere microbes that function as toxins as well as signaling molecules, thereby modulating plant immunity and shaping microbiome assembly (Jamil et al. 2022; Upadhyay et al. 2025). Secondary metabolites include phenolics, flavonoids, alkaloids, terpenoids, coumarins, benzoxazinoids, camalexin, and numerous related compounds (Table 3). In roots, many of these defence compounds are secreted into root exudates, where they suppress pathogens, attract beneficial microorganisms, and influence microbial community composition in the rhizosphere (Adediji and Babalola 2020; Pang et al. 2021; Korenblum et al. 2022; Ketehouli et al. 2025; Wankhade et al. 2025; Fan et al. 2025). Through this dual role as antimicrobial agents and ecological signals, secondary metabolites serve as critical mediators of plant-microbe interactions.

Among these, phenazines synthesized by *Pseudomonas spp* exhibit broad-spectrum antimicrobial activity and function as electron carrying molecules in microbial metabolic processes (Dundas and Dinneny 2022). Collectively, such compounds help suppress soil borne pathogens and can induce systemic resistance in plants (Martins et al. 2023b; Dubey et al.

2025). Siderophores represent another major class of microbial secondary metabolites that enhance plant iron nutrition while limiting pathogen growth through competitive iron sequestration (Jacoby et al. 2017a; Dundas and Dinneny 2022). These high-affinity iron chelating molecules enable bacteria to acquire iron from insoluble ferric forms commonly present in soils (Jacoby et al. 2017a). Plants can subsequently take up iron siderophore complexes through specialized transport systems, these mechanism particularly advantageous in alkaline soils where iron bioavailability is low (Jacoby et al. 2017a). The dual function of siderophores in nutrient acquisition and pathogen suppression makes them highly valuable traits for survival of Tissue culture plants (Jacoby et al. 2017a).

Volatile organic compounds (VOCs) produced by rhizosphere bacteria constitute another class of secondary metabolites involved in plant signaling. These compounds can promote plant growth, activate immune responses, and modulate stress adaptation mechanisms (Martins et al. 2023b). Bacterial VOCs such as 2,3 butanediol and acetoin have been shown to increase plant growth and stimulate stress tolerance pathways, and the precise molecular mechanisms underlying these effects remain partially understood (Martins et al. 2023b). Because VOCs can influence plant physiology without direct root contact, they represent particularly promising tools for facilitating the acclimatization of tissue cultured plants to *ex vitro* conditions (Martins et al. 2023b).

Rhizosphere microbes secrete low molecular weight organic acids like gluconic, citric, and malic acid, which chelate metal ions and solubilize insoluble phosphates and iron compounds in soil, making them available for plant uptake (Vyas and Gulati 2009). These acids lower soil pH locally and enhance mineral bioavailability, a key mechanism for overcoming nutrient deficiencies in acclimatizing of tissue cultured plants (Vyas and Gulati 2009). For instance,

Pseudomonas and *Bacillus* strains produce gluconic acid through glucose dehydrogenase, mobilizing up to 30-50% more phosphorus under Phosphorus deficient conditions (Suleman et al. 2018). Collectively, these metabolites alters root system architecture by stimulating lateral root formation, enhancing root hair development, and improving iron and phosphorus acquisition (Vacheron et al. 2013). Such functions are particularly critical for young tissue-cultured plantlets, which frequently encounter nutrient limitations and transplant shock during acclimatization (Chandra et al. 2010b).

Table 3: Microbial Biomolecules and Their Specific Effects on Acclimatization of Tissue cultured plants

Table 3

Biomolecules/ Biochemicals	Example Compounds	Mechanism of Action	Benefit for TC Plant	Referen ce
Phytohormones	IAA, Zeatin, GA3	Regulate division/elongati on	Enhanced architecture, shoot growth	(Castro Molina et al. 2025)
Enzymes	ACC Deaminase	Degrades ethylene precursor ACC	Reduced stress-induced senescence/inhibit ion	(Jha et al. 2021)

Volatiles	2,3-Butanediol, Acetoin	Signaling via gas phase	Induced Systemic Resistance (ISR), Growth	(Ryu et al. 2003)
	Catalase, Peroxidase Ascorbate Peroxidase	Neutralization of harmful Reactive Oxygen Species (ROS)	Mitigation of oxidative damage and improved survival under salt and heat stress	(Ahkami et al. 2017; Zia et al. 2021)
Antibiotics				
Organic Acids				
Secondary Metabolites				
Cell Lytic Enzymes				
Osmolytes				
Antioxidants				
Microbial Elicitors				

Iron Chelators				
ROS-Scavenging Enzymes				
Polymers	Alginate, Levan (EPS)	Soil aggregation, water retention	Protection from water loss, improved contact and enhanced microbial	(Scavino and Pedraza 2013)

Phytosequestration Polymers	Exopolysaccharides (EPS) Alginate, Levan	Biofilm formation and soil particle aggregation	Protection from desiccation and improved root surface contact	(Ahkami et al. 2017; Zia et al. 2021)
Metabolic Precursors	Tryptophan, Myo-inositol	Act as nutritional substrates or signaling precursors	Stimulation of microbial IAA production and nodule formation	(Agorsor 2026)

4. Rhizosphere engineering

Rhizosphere engineering is a comprehensive strategy designed to enhance crop productivity and agricultural sustainability by modifying the complex interactions within the soil surrounding plant roots (Dessaux et al. 2016; Ahkami et al. 2017; Zia et al. 2021). This approach focuses on optimizing the three core components of the root environment: the soil, the plant, and the microbial community (Dessaux et al. 2016; Ahkami et al. 2017; Zia et al. 2021). For instance, soil can be enhanced with materials like biochar to improve quality and water retention (Dessaux et al. 2016; Zia et al. 2021). Simultaneously, plants can be selected or engineered to release specific chemical signals and nutrients known as root exudates which attract and support beneficial microorganisms (Ahkami et al. 2017; Zia et al. 2021; Agorsor 2026). Introducing plant growth promoting rhizobacteria (PGPR) directly into the root zone helps plants acquire essential nutrients like nitrogen and phosphorus while providing protection against environmental stresses such as drought and high salinity (Ahkami et al. 2017; Zia et al. 2021). By tailoring these natural plant microbe interaction, rhizosphere engineering aims to create resilient agricultural systems that reduce the global reliance on chemical fertilizers and pesticides (Zia et al. 2021; Dries et al. 2021).

Modern strategies in rhizosphere engineering are increasingly moving toward the creation of synthetic microbial communities (SynComs), where multiple beneficial microbes are designed to work together as a group rather than acting alone (Ahkami et al. 2017; Agorsor 2026). This teamwork helps the microbes survive better in the soil and provides the plant with a wider range of benefits, such as improved resistance to diseases and better access to nutrient uptake (Ahkami et al. 2017; Zia et al. 2021; Agorsor 2026). Furthermore, scientists are using advanced genetic tools to help plants to communicate more effectively with these microbes by altering root signaling molecules and exudate profiles, ensuring the plant needs the right microbes at the right time (Dessaux et al. 2016; Ahkami et al. 2017). This promotes holobiont concept, where the plant and its entire microbial community are treated as a single superorganism that can be collectively engineered to create more productive and stress resistant crops (Dessaux et al. 2016; Ahkami et al. 2017).

4.1 Synthetic Microbial Community (SynComs)

Synthetic microbial communities, known as SynComs, are groups of two or more cultured microorganisms put together to mimic the natural structure and function of a microbiome (Shayanthan et al. 2022). They work by taking nature microbial complexity and shrinking it down into smaller, well-defined sets of microbial species. This makes them a kind of central strategy, helping scientists to connect the study of microbes with the large, complicated natural ecosystems. Therefore, high-throughput experimental methods are needed to construct SynComs by understanding their microbiological features, such as microbial library preparation in accordance to their genomes (Chakraborty 2023). Inside these communities, microbes follow a kind of division of labour,

where they interact to each other and share resources. This teamwork makes the entire community stronger against environmental changes and pathogen invasion.

5.2 Strategies of SYNCOM

To build these communities of microbes, scientists usually use one of two methods: a top down approach, which assembles SynComs from individual, well characterized microbial strains with known traits, functions, and interactions to achieve specific community level properties and works backward, or a bottom up approach, where they start with a complex natural microbial community and selectively simplifies it into a representative subset of taxa to replicate the diversity at specific composition, and to know the functionality of the original targeted microbiome (Sai et al. 2022). These design are also starting to add genetic diversity within each species, to know the stability and productivity seen in natural microbial systems. In the end, these purpose built microbial groups give scientists a useful tool to restore soil health, clean the environmental pollution, and protect plants from disease.

5.3 Strain Selection

The isolation selection assembly framework provides a structured strategy for designing customized SynComs (Sai et al. 2022). This approach begins with isolating a wide collection of microbial strains from ecologically relevant environments. Strain selection represents the foundational step in constructing effective synthetic microbial communities (SynComs) and requires integration of ecological principles and functional trait analysis to the understanding of community assembly works (Jacoby et al. 2017b; Martins et al. 2023b; Dubey et al. 2025). This process must balance multiple objectives, including preservation of functional diversity, promotion of community stability, optimization of root colonization efficiency, and compatibility with the target plant species. Functional trait screening is a critical step for identifying the strains with beneficial traits. Most plant growth promoting traits that include auxin production, ACC deaminase activity, phosphorus solubilization, nitrogen fixation, siderophore synthesis, and biofilm formation (Jacoby et al. 2017b; Martins et al. 2023b; Rigerte et al. 2025; Dubey et al. 2025). For stress tolerance applications, additional properties such as osmoprotectant synthesis, antioxidant activity, and stress responsive gene expression are evaluated and validated through *in vitro* assays (Yadav et al. 2024). Genomic analysis provides deeper insight into strain capabilities and potential interaction patterns, where whole genome sequencing can reveal genes associated with plant growth promotion, stress resilience, and metabolic production (Rigerte et al. 2025). Comparative genomics further enables identification of complementary metabolic pathways that support coexistence through cross feeding interactions (Jacoby et al. 2017b). Analysis of mobile genetic elements and CRISPR arrays can additionally indicate the likelihood of horizontal gene transfer or phage susceptibility, factors relevant to long term community stability.

5.4 SYNCOMS assembly

Principles of community assembly guide the selection of compatible strains for constructing stable and functional consortia (Hu et al. 2022). Key considerations include: (1) functional redundancy, incorporating multiple strains with overlapping roles to enhance resilience; (2) metabolic complementarity, selecting strains with distinct resource utilization profiles to minimize competition; (3) facilitative interactions, including strains that supply metabolites or services beneficial to other community members; and (4) Incorporation of keystone taxa, where the species whose presence affects the community's structure and function more than expected (Hu et al. 2022).

Traditional methods for studying microbial communities usually follow two types: first the holistic meta omics approaches, which provide broad data but lack the resolution needed to identify cause and effect relationship among the microbes, and second reductionist isolation based approaches, which often miss the complex interactions between species that are needed for community level functions to emerge (Ishizawa et al. 2025). Nowadays community assembly of microbiomes is well achieved using machine learning. These approaches are increasingly applied to predict strain combinations that yield stable and high-performing microbial communities (Ishizawa et al. 2025). These models leverage datasets linking community composition with functional outcomes to infer stability rules and optimize performance (Ishizawa et al. 2025). In some cases, computational predictions reveal non intuitive strain combinations that outperform communities designed individually through conventional ecological reasoning.

Determining appropriate SynCom complexity is another critical design parameter. While greater diversity often enhances functional redundancy and ecological stability, excessive complexity can hinder reproducibility, scalability, and mechanistic interpretation (Xu et al. 2025). Most successful SynComs comprise approximately 5-20 strains, balancing functional diversity with experimental and commercial feasibility (Xu et al. 2025; Zhang et al. 2026). The optimal level of complexity ultimately depends on the intended application, with more diverse consortia sometimes required to withstand unpredictable field environments.

5.5 Function Oriented SynCom Construction

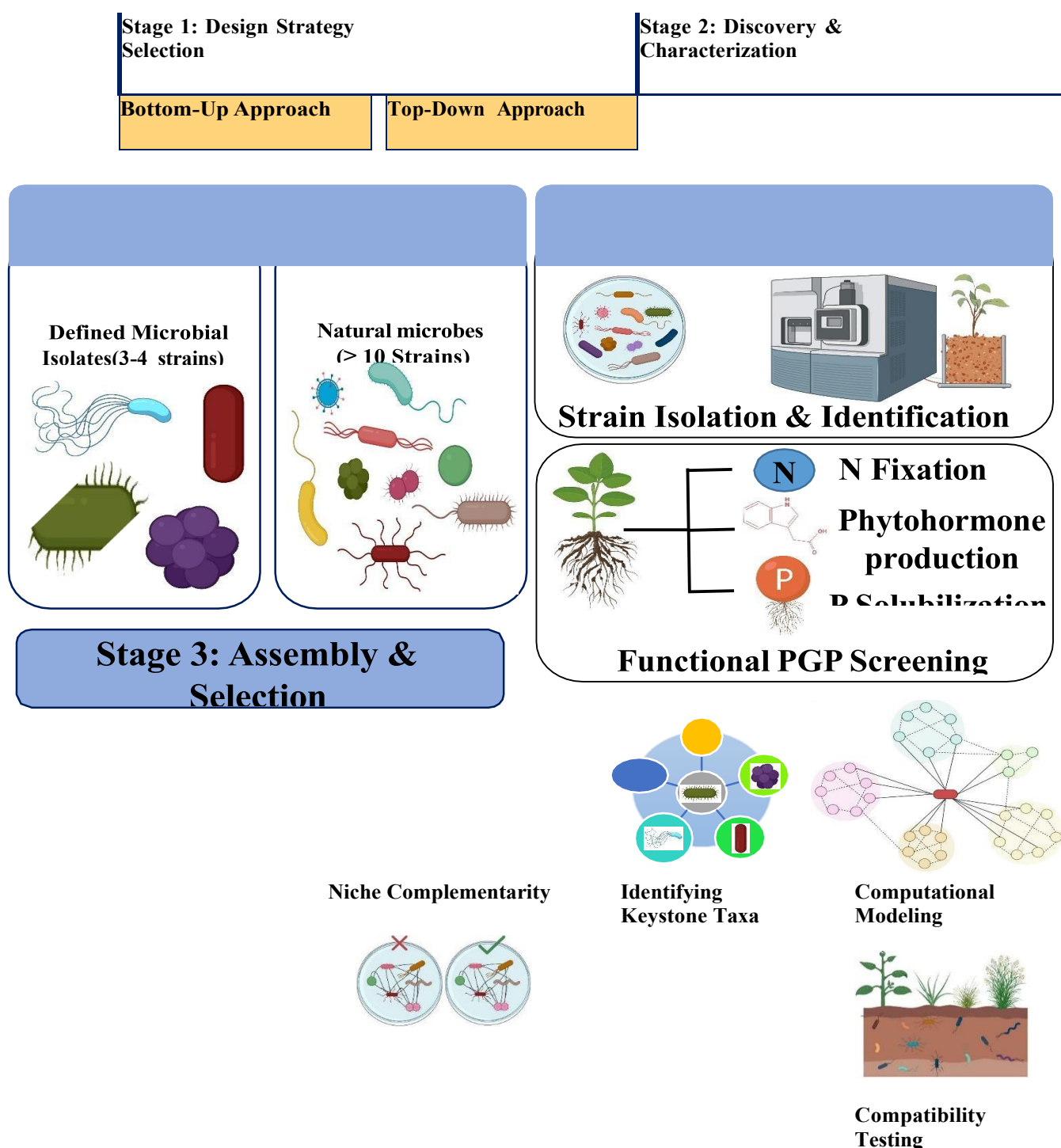
Function driven design focuses on constructing microbial communities that deliver specific beneficial traits designed to a defined application. This strategy begins with identifying target outcomes such as enhanced drought tolerance, improved nutrient uptake, and pathogen suppression, subsequently selecting strains that

collectively provide the required functional outcomes. An important principle of function driven design is the deliberate use of functional redundancy to enhance community resilience (Choi et al. 2026). Incorporating multiple strains capable of performing similar beneficial roles ensures sustained functionality even if certain microbial strains decline due to environmental fluctuations or competitive interactions. This functional overlap is particularly valuable under field conditions, where environmental variability can affect microbial persistence and activity (Jing et al. 2024; Choi et al. 2026).

Strains exhibiting multiple beneficial traits are especially advantageous for SynCom construction. For example, strains that produce both auxins and ACC deaminase provide complementary growth promoting effects, while strains capable of phosphorus solubilization and siderophore production can simultaneously address multiple nutrient constraints. Utilizing multifunctional strains supports functional diversity while maintaining an optimal number of community members (Rigerte et al. 2025).

Figure 1

Composing a Microbial Symphony



Stage 4: Validation



Green house Trials and controlled Assays

SynComs are tested on germ-free and soil grown plants to evaluate impact on plant biomass, nutrient uptake, and stress resilience.

Figure 1: Synthetic Microbial Communities (SynComs) for tissue culture plants go through four key steps. The researchers have selected two design approaches: the Bottom Up Approach involves the selection of a small community, generally a population of approximately 4 of the chosen beneficial microbes that can be cultivated in sterile tissue culture; and the Top Down Approach, reduces a microbial community one can find in nature to about more than ten functional members. At stage 2 helpful organisms are harvested from plants, tested for desirable characteristics like nitrogen-fixing ability, phosphorus solubilization, phytohormone production and pest control. At stage 3, the selected microbes are co-cultured to know if they could destroy their own species; they are also co-cultured with other microbes to determine if they could eliminate competition with other microbes; computer simulation models are then used to predict the co-culture results in order to identify certain key microbes whose extinction would affect the community. After construction, the SynCom is tested on tissue culture plants in a greenhouse or pot culture to assess plant growth and their ability to absorb nutrients and resist stress before it is tested on a larger scale.

6. Biotization

Biotization also known as biological hardening, is a process which involves introduction of beneficial microbes to tissue cultured plants to restore rhizosphere functions and improve survival rate, growth and disease resistance. While many traditional studies utilize a single, dominant mutualistic or endophytic microbe, modern research increasingly favors multi microbial consortia (SynComs) to leverage functional complementarity and synergistic effects (Figure 2). According to Indravathi (2024), biotization is a bio hardening technique characterized by the metabolic response of plant material to a microbial inoculum, which leads to physiological and morphological developments and increase resistance to biotic and abiotic stresses (Indravathi 2024). Soumare et al (2021) reported that this approach is often applied during micropropagation to minimize the need of costly chemical inputs and synthetic growth regulators. The aim of the process is to prepare young plants for the transition to natural environments, significantly improving their ability to survive the transplantation shock associated with the acclimatization phase. For example biotization of Grande Naine banana plants with *Methylobacterium salsuginis* TNMB03 significantly improved growth and survival during the transition to *ex-vitro* conditions. By colonizing plants as endophytes and epiphytes, the bacteria produce growth promoting phytohormones like cytokinins and auxins that enhance plant development and protect against abiotic stresses, including heat and direct sunlight. This effective biohardening allows plants to be transferred directly to open environments, potentially eliminating the need for traditional, labour intensive acclimatization process (Pushpakanth et al. 2021).

6.1 Biotization in acclimatization of TC plants

Biotization, of Tissue cultured plants build resilience in micropropagated plantlets against the environmental stresses of the *ex vitro* transition. In tissue cultured sugarcane, the application of strains such as *Bacillus* 182 and *Pseudomonas* 336 has been shown to significantly improve survival rates by as much as 20% under high stress conditions (Michavila et al. 2022). For tissue cultured tea plants, the introduction of antagonistic rhizosphere bacteria of *Bacillus subtilis*, *Bacillus* sp., and *Pseudomonas corrugata* provides a first line of defense against fungal pathogens such as *Fusarium oxysporum*, boosting survival from roughly 50% up to 100% depending on the season (Pandey et al. 2000). Additionally in banana micropropagation, biohardening with combinations of *Pseudomonas fluorescens* (Pfl, CHA0) and endophytic *Bacillus species* (EPB22, EPB5) significantly reduces the incidence of Banana bunchy top virus (BBTV) and *Fusarium* wilt by inducing systemic resistance (ISR) and upregulating defense related enzymes like peroxidase and phenylalanine ammonia lyase (PAL) (Harish et al. 2008; Kavino and Manoranjitham 2018). Furthermore, the endophyte *Bacillus velezensis* YEBBR6 has been identified as a unique candidate that suppresses *Fusarium* wilt by 100% through hyper parasitism and the production of antifungal biomolecules (Harish et al. 2008). For tissue cultured sugarcane, the integration of biopriming with

Gluconacetobacter diazotrophicus and phenolic metabolites has resulted in a nearly 99% recovery rate of plantlets with well-developed root systems (Bernal et al. 2008).

Castro Molina et al. (2025) demonstrated that biotization of *in vitro* potato seedlings with *Azospirillum brasilense* Ab-V5 increases survival rates by 1.3 fold and tuber production by 1.9 fold. These benefits are driven by the synthesis of growth-promoting IAA ($48.3 \mu\text{g mL}^{-1}$) and GA3 ($13 \mu\text{g mL}^{-1}$), which regulate plant morphology, and salicylic acid (SA), which triggers systemic acquired resistance (SAR) against environmental stress. As these outcomes are strain specific, re-inoculation of plant at key growth stages was recommended to maintain colonization which, potentially increased total biomass up to 5 fold over un-inoculated controls (Castro Molina et al. 2025).

Biotization of tissue cultured banana plants with *Bacillus spp.*, arbuscular mycorrhizal fungi (AMF), and *Trichoderma spp.* during acclimatization and potting (Mwangi et al. 2013), resulted in improved survival rates during the hardening phase. However, the effectiveness of specific inoculants varied according to soil type, *Bacillus sp.* performed optimally in Vertisol soils while combined application of mycorrhiza and *Trichoderma* proved more effective in Rhodic Ferralsol. Biotization of *Mentha spicata* with *Trichoderma asperellum* and *Bacillus subtilis* during the *in vitro* rooting phase ensured complete root colonization, significantly increased *ex vitro* survival rates from 75% to over 93%. These pre-colonized plants demonstrated enhanced tolerance to environmental fluctuations and achieved higher dry biomass than untreated controls. Additionally, these endophytes protected against Rhizoctonia infection by outcompeting the pathogen and activating plant defenses, ultimately promoting superior growth and development (Castro-Restrepo et al. 2022).

6.2 Biotization in TC plant development

Biotization significantly enhances the physiological quality and growth of tissue-cultured plants by mimicking natural beneficial interactions. In tissue cultured banana, inoculation with *Methylobacterium sp.*, such as *M. salsuginis* and *M. radiotolerans*, leads to substantial increases in pseudostem height, leaf area, and biomass due to the bacterial synthesis of vital phytohormones like cytokinins (zeatin, kinetin) and auxins (IAA) (Senthilkumar et al. 2021). While the use of Arbuscular Mycorrhizal Fungi (VAM), specifically *Glomus fasciculatum*, is highly effective for nutrient acquisition, allowing plantlets to accumulate major nutrients like nitrogen, phosphorus, and potassium (NPK) more efficiently (Mandhare and Suryawanshi 2005). Additionally, *Bacillus velezensis* YEBBR6 had been shown to increase leaf area and improve photosynthetic rates biohardening of *in vitro* tissue cultured banana plant (Saravanan et al. 2022). Other bacteria, such as *Paenibacillus glucanolyticus*, *Curtobacterium pusillum*, and *Methylobacterium extorquens*, are used during the multiplication stage of *in vitro* culture to increase the number and length of axillary shoots in ornamental plants like Chrysanthemum and Gerbera (Cantabella et al. 2022).

Additionally biotization enhances stomatal function to mimic greenhouse hardened plants, leading to better water management. Furthermore, it triggers systemic resistance by increasing defense enzymes like phenylalanine ammonia lyase(PAL), which protects against pathogens and enhances tolerance to osmotic stress (Nowak 1998b). Vestberg (1992) demonstrated that the inoculation of micropropagated strawberry plantlets with arbuscular mycorrhizal fungi (AMF), such as *Glomus intraradices*, at the beginning of the acclimatization stage provided critical physiological and structural support. This early symbiotic establishment facilitated rapid colonization of the root cortex, effectively compensating for the anatomical deficiencies *in vitro* formed roots, including poor suberization and the absence of a functional exodermis. The resulting external hyphal network served as a functional support for a mature root system, significantly expanding the absorptive surface area for water and nutrient uptake while simultaneously protecting the plant from root necrosis (Vestberg 1992).

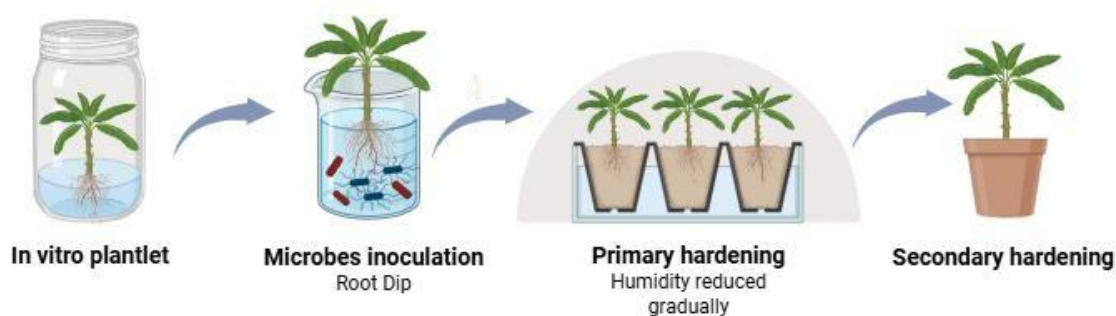


Figure 2

Figure 2: The workflow of biotization of tissue culture plantlets prior to hardening.

7. Future Perspectives

The use of SynComs in crop production has shown considerable value in improving nutrient uptake, promoting plant growth, and enhancing tolerance to biotic and abiotic stresses such as drought, salinity, and pathogen attack. However, so far there are no studies specifically dedicated to the biotization of micropropagated plants using

SynComs. Extending this approach to tissue culture could create new opportunities to improve plant establishment and acclimatization, as SynCom treated plants may exhibit higher survival rates, improved rooting, and greater resilience during the transition from *in vitro* to *ex vitro* conditions. Hence, developing crop and genotype specific SynComs for tissue cultured plants may emerge as an important future strategy for enhancing plant vigor, survival, and stress tolerance during acclimatization.

8. CONCLUSION

Engineering rhizosphere microbiomes represents a transformative strategy for improving the establishment of tissue cultured plants during their transfer from lab to field conditions. Biotization with beneficial microorganisms enhances plant tolerance to biotic and abiotic stresses during acclimatization, thereby reducing transplant shock, shortening the hardening period, and significantly improving survival rates. This acceleration of the hardening process reduces the time required for plantlets to adapt from *in vitro* to *ex vitro* condition, ultimately increasing the efficiency and economic viability of micropropagation systems. Although the benefits of biotization are well known, using designed SynComs in micropropagation is still largely unexplored. Based on this, the integration of Synthetic Communities (SynComs) within micropropagation workflows presents a significant opportunity to enhance the commercial scalability and biological performance of clonally propagated plants. Incorporating beneficial microbial inoculants at critical stages of the micropropagation including rooting, acclimatization, and pre transplant hardening can adopt plantlets for improved nutrient uptake, hormonal balance, and stress resilience prior to field transfer. Looking ahead, standardizing microbiome assisted micropropagation protocols across high value crops will be essential to achieving consistent, reproducible outcomes at commercial scale. Furthermore, coupling microbiome biotization with optimized tissue culture media and controlled environment acclimatization chambers could redefine quality for micropropagated plantlets, ultimately increasing the productivity and sustainability of modern plant propagation industries.

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