CHAPTER V
DISCUSSION

Bacillus velezensis is recognized for its diverse applications as a PGPB and a plant-growth-promoting rhizobacterium (PGPR) (Bagheri et al., 2022; Bai et al., 2023; Balderas-Ruíz et al., 2020). *B. velezensis* has been identified as a PGPB in cannabis, similar to in this study, which discovered the highest S141 copy number in surface-sterilized tissues and leaves, followed by the stems and roots of inoculated cannabis. No S141 was present in the non-inoculated group. This result highlights that S141 might be an endophytic bacterium in cannabis (Figure 8). Several strains of *B. velezensis* are known for their capacity to enhance plant growth through various mechanisms. *B. velezensis* FZB42, renowned for producing phytohormones such as indole-3-acetic acid (IAA), improves nutrient uptake, leading to increased biomass and stress tolerance in crops like *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) (Chowdhury et al., 2015; Liu et al., 2017). *B. velezensis* 83 promotes maize growth by stimulating root development through phosphate solubilization, auxin production, and volatile organic compound emissions, thus boosting overall plant vigor (Balderas-Ruíz et al., 2020).]. Moreover, *B. velezensis* BS1 has demonstrated biocontrol efficacy against fungal pathogens in pepper plants, contributing to enhanced plant health and growth by reducing disease stress (Shin et al., 2021). S141 is seen as a promising plant-growth-promoting rhizobacterium with multifaceted benefits for agricultural systems. It enhances plant growth, nodulation, and nitrogen fixation efficiency, particularly in collaboration with crops like soybean, highlighting its potential as a beneficial tool in sustainable agriculture (Sibponkrung et al., 2020). Additionally, S141 showcases the capacity to boost arbuscular mycorrhizal symbiosis, increasing nutrient absorption and usage in host plants. By activating key plant marker genes linked to mycorrhizal symbiosis and upregulating the genes essential for nutrient absorption, S14 contributes to improved plant performance and resilience.

Its effect on the cost benefit balance in mycorrhizal symbiosis underscores its complex interactions within plant–microbe systems (Kiddee et al., 2024). In this study, S141 inoculation substantially enhanced cannabis growth, particularly at an optimal concentration of 10^6 CFU/ml, as demonstrated by increased trunk circumference, height, chlorophyll content, and dry weight (Figure 9). Furthermore, greenhouse experiments with four separate soil conditions, including soils treated with boiled water and untreated soils, combined with either normal or low fertilizer levels, validated these findings. The results showed the practical benefits, such as improved growth parameters and reduced fertilizer needs, of treatments with this bacterium (Figure 10). These findings emphasize the potential of *B. velezensis* S141 to advance cannabis cultivation practices, promote cannabis growth, encouraging further exploration of its action mechanisms and optimization for extensive agricultural applications.

Transcriptomes have been utilized in cannabis to decipher how the plant responds and defends itself against biotic and abiotic stresses (Balthazar et al., 2020; Huang et al., 2019; Miotti et al., 2023). This study found transcriptomic analysis useful in identifying the plant genes associated with S141 inoculation (Figure 11). The GO term analysis showed that the upregulation of biological processes such as metabolic process, cellular process, localization, and biological regulation was higher than downregulation following *B. velezensis* inoculation. This suggests a connection to the network of metabolic pathways responsible for growth, development, and environmental responses (Liao et al., 2023). Consequently, differential gene expression observed in S141-treated *C. sativa* might be linked to its enhanced growth rate. Pathway analysis through the Kyoto Encyclopedia of Genes and Genomes (KEGG) highlighted several metabolism-related processes, including phenylpropanoid biosynthesis, plant-pathogen interactions, and plant hormone signal transduction. Previous studies have shown that transcription factors such as MdMYB88 and MdMYB124 regulate the accumulation of phenylpropanoid metabolites by modulating MdCM2 expression, aiding plants in combating pathogens and withstanding drought conditions (Geng et al., 2020). Effectors in plant–pathogen interactions operate in key ways: they break through physical barriers to invade the plant, create an environment that supports their survival inside the plant, and even employ tactics to evade or deceive plant defenses, while weakening the plant's immune responses (S. Zhang et

al., 2022). Our study observed such behaviors in plant–pathogen interactions after inoculation with the bacterium, which suggests that these interactions might develop and respond to the environment of the organismic system of plants. In the realm of plant hormone signal transduction pathways, membrane or transmembrane receptors display specificities and diversities. Acting as gateways, these receptors play an essential role in recognizing various hormones and transmitting signals into the cell (Song et al., 2017). Recent studies showed that the upregulation of phytohormone signal transduction is crucial in the response of Jerusalem artichoke (*Helianthus tuberosus* L., (Asteraceae)) seedlings to salt stress. The genes studied included those for abscisic acid, auxin, ethylene, and jasmonic acid (Yue et al., 2022). Plant growth regulators have been studied for their ability to enhance the growth and yield of rice under high-temperature conditions both day and night, with the most significant production found via hormone treatments that boosted photosynthesis (Fahad et al., 2016). Our results suggested that plant hormone signal transduction was represented by KEGG pathways during *B. velezensis* inoculation in *C. sativa*. This implies that biological processes related to gene metabolism, previously identified as pivotal in growth pathways, could be highly beneficial for plant growth in conjunction with plant–pathogen interactions and plant hormone processes. (Figure 14).

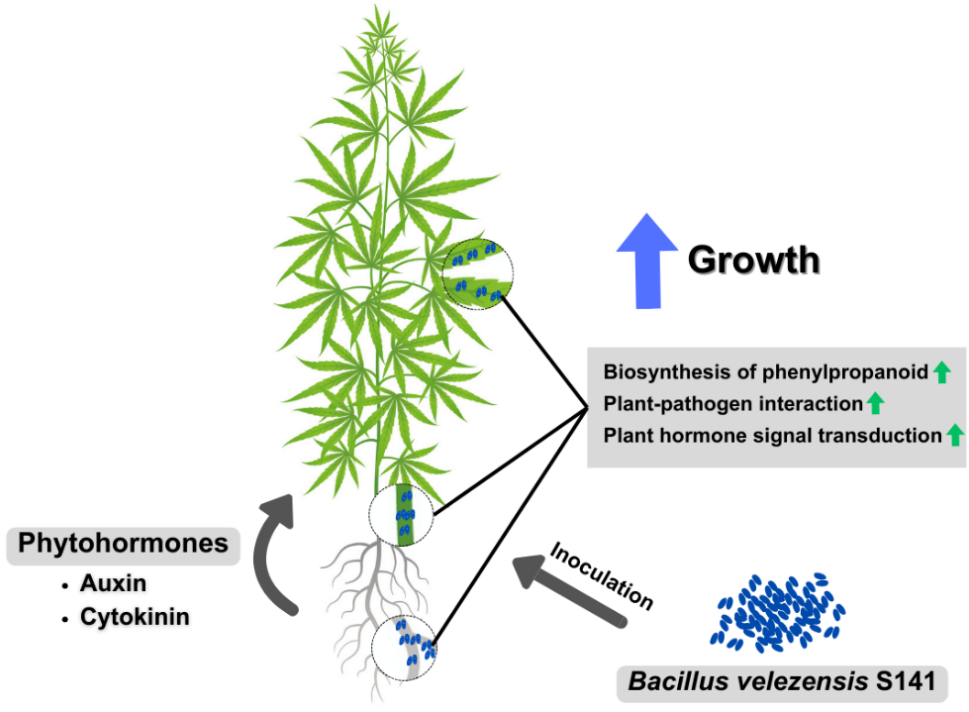

Figure 14. Schematic overview of mechanisms of cannabis growth promotion by *Bacillus velezensis* S141. S141, an endophytic cannabis bacterium, promotes cannabis growth by producing phytohormones and triggering genes involved in the biosynthesis of phenylpropanoid, plant–pathogen interaction, and plant hormone signal transduction pathways. This figure was created using https://www.canva.com/ (accessed on 21 August 2024).

In this study, we used qRT-PCR to explore the expression profiles of 18 genes related to cannabis growth (Figure 12), with a particular focus on their modulation by phytohormones involved in developmental processes. Genes such as SAUR50, IAA2, and ARR5 showed significant upregulation across all tissues (root, stem, and leaf). Previous studies have shown that auxins like IAA2 are essential in root and shoot development by regulating cell elongation and differentiation (Wu et al., 2017). SAUR proteins, such as SAUR50, have been associated with auxin-mediated growth responses, suggesting their conserved role across plant species (Ren & Gray, 2015). The observed upregulation of these genes aligns with their established roles in enhancing vegetative growth, a key factor in optimizing cannabis cultivation and biomass production. Genes such as UDP, CBD, CBL120, ARR12, ARR5, and CRF5 displayed tissue-specific expression, with predominant activity in stems and leaves, reflecting their roles in regulating growth pathways. For instance, cytokinin-responsive genes like ARR5 and ARR12 are implicated in shoot branching and delaying leaf senescence (Mason et al., 2005), potentially impacting cannabis plant architecture and yield. The upregulation of CBD-related genes in the stems aligns with the biosynthesis of cannabinoids, essential compounds with pharmaceutical relevance, thus emphasizing the economic significance of understanding their regulatory mechanisms in different plant tissues (Andre et al., 2016).]. Comparative analyses with previous studies emphasize both the evolutionarily con-served and divergent regulatory mechanisms of phytohormone-responsive genes across plant species. Studies on germinlike proteins (e.g., GLP2-1) involved in cell wall modification and growth regulation further highlight their conserved roles in hormonal signaling pathways affecting plant architecture and development (Cosgrove, 2018; Takahashi et al., 2018). The observed downregulation of genes like GTLS and LRLK4 suggests their potential roles in modulating receptor-

mediated signaling pathways and stress responses, indicative of complex hormonal crosstalk governing growth and development in cannabis and related species (Afzal et al., 2016; Gou et al., 2010). These findings underscore the intricate interplay between hormonal signals and gene regulation, emphasizing the importance of understanding these mechanisms for optimizing crop productivity and metabolic pathways in cannabis cultivation. Future research could examine in greater detail the specific molecular interactions and signaling pathways involved in phytohormone-mediated growth processes, thereby facilitating targeted breeding strategies and biotechnological advancements in cannabis agriculture.

The roles of the genes involved in the production of plant hormones, auxin and cytokinin, in bacteria are pivotal for understanding and optimizing plantmicrobe interactions. Genes such as *yhcx*, *IPyAD*, and *dhaS*, which are integral to auxin biosynthesis, enhance plant growth by promoting root elongation, nutrient uptake, and stress resistance (Lee et al., 2019; Q. Zhang et al., 2022). Likewise, cytokinin biosynthesis genes like IPT and IPI contribute to shoot growth, cell division, and delay in leaf senescence (Glanz-Idan et al., 2022; Kant et al., 2015). Mutations in these genes can drastically reduce hormone production, leading to reduced bacterial colonization, plant growth promotion, and disease resistance. In prior studies, co-inoculation of *Bradyrhizobium diazoefficiens* F (*Xanthobacteraceae*) strain USDA110 with S141Δ*yhcx* and S141Δ*IPI* reduced the number of nodules, indicating the significant impact of *yhcx* and *IPI* on promoting soybean growth (Sibponkrung et al., 2020). In this study, we elucidated the distinct roles of the genes involved in plant hormone production in cannabis growth via the investigation of S141 and its mutant strains. Significant differences in growth parameters emerged between the inoculated and control groups. Specifically, both wild-type S141 and mutants possessing *dhaS*, *yhcx*, and *IPI* genes exhibited marked increases in root length, plant height, root dry weight, total dry weight, and the health index (HI), underlining the importance of these genes in promoting robust cannabis growth (Figure 13A–F). Conversely, mutants lacking *IpyAD* and *IPT* genes displayed no significant improvements in these metrics compared to the controls, implying a crucial role of *IpyAD* and *IPT* in mediating cannabis growth promotion. These findings contribute to our understanding of how specific genetic

elements in S141 enhance plant growth, potentially through the modulation of the hormone signaling pathways essential for developmental processes in cannabis.

This study offers substantial evidence of the growth-promoting effects of S141. However, further research is necessary to thoroughly understand its mechanisms of action and to optimize its use in cannabis cultivation. Future research could concentrate on understanding the distinct metabolic pathways and signaling mechanisms engaged in plant–microbe interactions, as well as investigate potential synergies with other beneficial microorganisms or agricultural inputs. Moreover, conducting field trials over several growing seasons and under varied environmental conditions could help confirm the effectiveness and consistency of S141 inoculation across different cannabis cultivars and cultivation practices.