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***Trichoderma* sp. strain AM6 whole-genome guided untargeted metabolomics: Terpenoid backbone synthesis and modulation of VOCs in tea (*Camellia sinensis*)**

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Abstract:

Tea, the widely consumed beverage, is not only a cultural heritage but also considered as a cash crop for its bioactive components. Among them, terpenoids and other volatile organic compounds (VOCs) are synthesized through specialized metabolic pathways, which significantly contribute to distinct flavor and aroma as well as enhanced therapeutic potentials of tea. *Trichoderma* species, the well established biocontrol agents are plant growth promoters too. Their potential effectiveness in modulation of plant secondary metabolism, particularly terpenoid biosynthesis of *C. sinensis* remains unexplored. Integrating a comprehensive multi-omics strategy, the genomic and metabolomic intricacies of *Trichoderma* sp. AM6 and its interaction with *C. sinensis* were unraveled. Whole genome sequencing revealed a compact yet intricate genomic architecture with a size of 39.91 Mbp comprising 446 contigs organized into 406 scaffolds, with 98.8% completeness. Metabolic pathway analysis from genome data indicates exclusive reliance on the mevalonate pathway for terpenoid biosynthesis in *Trichoderma* sp. AM6, unlike *C. sinensis*, which utilizes both the mevalonate and non-mevalonate (MEP/DOXP) pathways for the production of terpenoids. Untargeted LC-ESI-MS/MS analysis of *Trichoderma* sp. AM6 identified a total of 11,841 secondary metabolites, including 34 monoterpenoids, 72 diterpenoids and 76 sesquiterpenoids, emphasizing its potential to contribute to specialized metabolite pools. Comparative phylogenomics positioned it as a phylogenetically distinct species with unique adaptive traits. Untargeted GC-MS reveals the highest abundance of volatiles from microbial consortia treated (T5) sets followed by only *Trichoderma* treatment (T2) in comparison with uninoculated control (T1). Terpenoid transcripts of *C. sinensis* from the plant-microbe consortium assembly sets (T1, T2, T5) illuminate upregulation of genes assigned to 3-hydroxy-3-methylglutaryl coA reductase (HMGCR), the key enzyme in the mevalonate pathway and downregulation of genes assigned to 1-Deoxy-D-xylulose 5-phosphate (DXS), indicating a metabolic shift towards more mevalonate pathway activity influenced by the novel *Trichoderma* strain itself and in combination with other tea root associated microbes.

Keywords: *Trichoderma*; Whole genome; metabolites; terpenoids biosynthesis; Tea VOCs

Introduction:

Tea, the second most widely consumed beverage followed by water, is made from *Camellia sinensis* plant (Khan & Mukhtar, 2013; Bag et al., 2022a). Apart from its traditional significance, the tea plant is considered to be the most important cash crop or commercial crop contributing to its role in rural development, poverty reduction, and food security in exporting and developing countries as reported by the Food and Agriculture Organization (FAO) of the United Nations (Jiang et al., 2019; Zhau et al., 2020). The celebration of International Tea Day on 21 May from the serene tea gardens of Darjeeling to the bustling tea houses in Beijing shows the global importance of tea (Pan et al., 2024). The plant formerly known as *Camellia sinensis*, or tea, is a member of the *Theaceae* family and is typically grown in tropical and subtropical regions (Drew, 2019; Wang et al., 2022). There are two main varieties of *C. sinensis*: *C. sinensis* var. *assamica*, found mainly in India and Sri Lanka, is recognized for its robust growth and larger leaves, while *C. sinensis* var. *sinensis* is found in China and Japan and has smaller leaves and a compact growth habit (Shafiq et al., 2024).

Two components make up the entire flavor of tea: aroma, made up of volatile compounds, and taste derived from non-volatile compounds. In addition to various flavors and cultural value, *C. sinensis* possesses several health benefits because of the high concentration of bioactive compounds embedded within it (Majumder et al., 2022). Terpenes, also known as terpenoids, are considered the most diverse groups of bioactive compounds consisting of 40,000 structurally diverse individual compounds that contribute to about 60% of the known natural compounds (Thoppil & Bishayee, 2011). Terpenes are classified based on the organization pattern and the number of isoprene units. These classes include hemiterpenoids, which have one isoprene unit (C₅), monoterpenoids with two units (C₁₀), sesquiterpenoids with three units (C₁₅), diterpenoids with four units (C₂₀), sesterterpenoids with five units (C₂₅) and triterpenoids with six units (C₃₀) (Smanski et al., 2013). They play an extensive variety of functions in both the plant-microbe as well as plant-plant interactions with their biotic and abiotic surroundings and in fundamental physiological processes including plant growth and development as well as regulation of resistance mechanisms (Zhou et al., 2000; Zhu et al., 2019; Bag et al., 2022b). According to reports, monoterpenoids and triterpenoids are being studied intensively as anticancer drugs, as they inhibit the growth of cancer cells by either influencing the differentiation of tumor cells and causing death, or by preventing the

angiogenesis, invasion, and metastasis of tumors (Cox-Georgian et al., 2019; Kamran et al., 2022; Lu et al., 2024).

Several parameters including seasonal variations, climate change, cultivation techniques, and leaf maturity significantly impact the composition of tea bioactive compounds (Zaman et al., 2023; Chen et al., 2025). A systematic review performed by Ahmed et al., 2019 reported that the phenolic concentrations or the bioactivity of *Camellia sinensis* tend to decrease during the seasonal transition period particularly, from spring or first flush to other seasons with 78% of studies (14 out of 17) confirming the trend. The plucking seasons that occur throughout the year are referred to as flushes, which also significantly impact tea varieties' flavors and aromas (Owuor et al., 1990; Li et al., 2023). Continuous cultivation of tea simplifies production and ensures a steady tea supply but possesses serious soil health and genetic diversity challenges (Jibola-Shittu et al., 2024). Several issues like erosion, compaction, and nutrient loss can lead to soil degradation, moreover, inadequate soil management worsens soil fertility, affecting plant nutrition and quality (Islam et al., 2021). Monoculture systems in continuous cultivation are less resilient to pests and diseases, causing uniform flavors in tea.

Microorganisms present within the soil play a great role in nutrient cycling, impacting soil health and nutrient availability (Bag et al., 2022c). The potential of soil microorganisms for enhancing tea yield as well as quality for sustainable production was addressed by Jibola-Shittu et al., 2024. Plant growth-promoting fungi (PGPF), such as ectomycorrhizal fungi (ECM) and arbuscular mycorrhizal fungi (AMF), develop symbiotic interactions with plant roots to increase photosynthetic efficiency, nutrient uptake, and stress tolerance. With more than 200 species identified, the genus *Trichoderma*, a family of fungi belonging to the *Hypocreaceae*, is renowned for its exceptional adaptability and versatility. These filamentous fungi, found worldwide in diverse soil ecosystems, function as endophytes that reside within root tissues or colonize root surfaces, playing vital roles in ecological processes and agricultural practices (Samanta, 2015; Mukherjee et al., 2022). Certain microorganisms added to agricultural systems can cause stress responses in plants, which alters the profiles of volatile organic compounds (VOCs). The phenomenon referred to as 'microbial-induced volatile modulation' has garnered interest due to its possible uses in improving the resilience and quality of crops (Lui et al., 2015). Intriguingly, *Trichoderma* species are capable of producing and emitting beneficial compounds, particularly VOCs that possess multifaceted mechanisms involved in biological

control, inducing defense responses and plant growth promotion (Bae et al., 2009; Majumder et al., 2022;).

The ability of *Trichoderma* species through mechanisms such as competition, mycoparasitism, and the production of antifungal metabolites has been well studied till now (Yao et al., 2023; Saravanakumar et al., 2016). Additionally, their roles have been also studied in plant growth promotion and synthesis of important secondary metabolites, like flavonoids, in various plants, including tea (*Camellia sinensis*) (Ortega-García et al., 2015; Shang et al., 2020; Woo et al., 2023; Liu et al., 2023). However, the majority of the current research is focused on specific metabolites of *Trichoderma*-plant interactions, leaving a significant gap in understanding the broader spectrum of its synergistic potential with other plant-associated beneficial microbiomes. In particular, the synergistic interactions between *Trichoderma* sp. AM6 and synthetic microbial consortia designed to mimic beneficial tea root microbiomes remain unstudied. The ability of *Trichoderma* species to foster and regulate the biosynthesis of other crucial phytochemicals, such as terpenoids in tea plants, are yet to be fully explored. Our study aims to fulfill the research gap by exploring the ability of *Trichoderma* sp. AM6 to modulate terpenoid and VOC biosynthesis in tea seedlings. Novel insights into the multi-purpose role of *Trichoderma* sp. AM6 has also been explored through whole genome analysis. Alongside, transcriptomics and metabolomics studies had also been incorporated aiming to provide a comprehensive understanding of the underlying pathways modulated by *Trichoderma* sp. AM6 and synthetic microbial consortia. Our study using a multi-omics approach offers novel insight into a hitherto unexplored area of *Trichoderma* study.

2. Methodology:

2.1. DNA Isolation & Sequencing:

Culture medium used for the growth and maintenance of the *Trichoderma* sp. AM6 isolate was Potato Dextrose Agar (PDA). Genomic DNA of the aforesaid fungal isolate was extracted using a column-based DNA extraction kit (HiPurA® Fungal DNA Purification Kit; Himedia Cat. No. MB543) as per the suggested protocol by the manufacturer. The quality and quantity of the extracted genomic DNA were determined using the Micro-volume Spectrophotometer

(Eppendorf). Extracted DNA was sent for library preparation and whole genome sequencing using Illumina NovaSeq 6000 (Modi et al., 2021).

2.2. Genomic insights of *Trichoderma* sp. AM6:

2.2.1. Sequence Quality Assessment & Adapter Removal:

Provided raw sequence files were of .fastq format. As it was a paired-end run, the quality of both forward (R1) and reverse read (R2) were analyzed using FastQC version 0.12.1 (Andrews, 2010). Trimgalore version 0.6.7 (Krueger et al., 2021) was utilized with default parameters in order to trim the raw reads and removal of the adapter present within the sequence read file shown in [figure S3](#). Trimmed reads were again subjected to quality assessment using FastQC version 0.12.1.

2.2.2. Assembly and Functional Annotation of Genome:

The trimmed raw reads were assembled using spades version 3.15.4. (Bankevich et al., 2012). Genome completeness was assessed using Benchmarking Universal Single-Copy Orthologs (BUSCO) version 5.8.0. (Manni et al., 2021) with ascomycota_odb10 lineage datasets. For a detailed annotation of *Trichoderma* sp. AM6, assembly file (.fasta) was submitted to the Joint Genome Institute (JGI) pipeline which has a lot of quality control metrics, manual inspection, and community curation of predicted annotations accessible through JGI MycoCosm (Grigoriev et al., 2014). The method for transporter prediction was blastp against TCDB (<https://tcdb.org>) with E-value cutoff 1e-05 and alignment coverage cutoff 50% both for query protein and database. The standard operating procedure (SOP) for the annotation of fungal genome through the JGI automated pipeline is available at <https://mycocosm.jgi.doe.gov/programs/fungi/FungalGenomeAnnotationSOP.pdf>. Annotated files were downloaded, extracted and subjected to further visualization. The terpenoid backbone biosynthesis pathway (MAP00900) was comprehensively analyzed.

2.2.3. Prediction of Secondary Metabolite Gene Clusters:

AntiSMASH fungal version or fungiSMASH (<https://fungismash.secondarymetabolites.org/>) web version was used for the prediction of secondary metabolite biosynthetic gene clusters. A genome sequence file (.fasta) was submitted as data input. Detection strictness was set to be relaxed to detect well-defined clusters containing all required parts. Extra features including ClusterBlast, SubClusterBlast, KnownClusterBlast, MIBiG cluster comparison, Pfam-based GO term annotation, ActiveSiteFinder, and RREFinder were turned on and submitted (Blin et al., 2023).

2.2.4. Comparative Genomics and Phylogeny Analysis:

As of 01.09.2024 only 4 *Trichoderma* reference genomes were available with complete assembly level. *Trichoderma atroviride* P1 (ASM2064779v1) (Li et al., 2021), *Trichoderma asperellum* FT101 (ASM2064786v1) (Li et al., 2021), *Trichoderma simmonsii* GH-Sj1 (ASM1956561v1) (Chung et al., 2021), *Trichoderma semiorbis* FJ059 (ASM2004594v2) (Li & Liu, 2022) belonging to those reference genomes were subjected to sequence data collection from NCBI database. OrthoVenn3 (<https://orthovenn3.bioinfotoolkits.net/>) web server was employed to compare the orthologous protein clusters of above mentioned *Trichoderma* species along with pre-existing genome data of *Trichoderma harzianum*, *Trichoderma reesei* and *Trichoderma virens*.

2.3. Metabolomic insights of *Trichoderma* sp. AM6:

2.3.1. Extraction of Fungal Secondary Metabolites:

Liu and Liu method with some modifications was followed to extract secondary metabolites of *Trichoderma* sp. AM6 (Liu & Lui, 2018). The above-mentioned fungal strain was primarily grown on a solid medium (PDA) for 8 days. Upon cutting into small pieces (~ 8 mm) it was transferred aseptically into a 2 L Erlenmeyer flask containing 1000 mL of sterilized Potato Dextrose Broth (PDB). Therefore, it was incubated at 26 ° C on a BOD shaker (Infors-HT) at 150 rpm for 7 days, and for the rest of 5 days it maintained the same condition but without shaking. After a total incubation period of 12 days liquid culture and mycelial matrix were separated using autoclaved grade 1 Whatman filter paper. 40 mL of filtrate was taken into a 100

mL centrifuge tube, and an equal volume of ethyl acetate (HPLC grade) was added. Upon vigorous vortexing, it was allowed to settle down at room temperature for phase separation. The mentioned process repeated iteratively until the last volume of filtrate was completely collected. The upper layer (organic phase) was taken out, filtered and concentrated in a vacuum concentrator (Eppendorf). The obtained crude extract was subjected to LC-ESI-MS/MS analysis.

2.3.2. LC-ESI-MS/MS Metabolite Profiling:

The LC-ESI-MS/MS analysis was carried out in positive ion mode and had a mass range of 50–2000 m/z. Agilent SB-C18 columns were used to separate the compounds. As binary mobile phases, solvents A (H₂O with 0.1% formic acid) and B (acetonitrile with 0.1% formic acid) were used. The gradient flow rate was set at 0.4 mL/min for 12 minutes. A gradient of 95% A and 5% B for 0–2 minutes, 95%→5% A and 5%→95% B for 2–10 minutes, and 50% A and 50% B for 10–12 minutes was used. Using an auxiliary solvent management system, 100% isopropyl alcohol was provided at a rate of 10 µL/min. For internal calibration, leucine enkephalin was utilized (Wang et al., 2021).

Obtained list of identified metabolites were subjected to standard InChI key conversion with the help of OPSIN (Open Parser for Systematic IUPAC nomenclature) web service (<https://www.inchi-trust.org/inchi-post/iupac-name2pubchem/>). As the list of untargeted metabolite was huge we took help of smart spreadsheet available at https://docs.google.com/spreadsheets/d/1CJcwyFQ7FF77HAGGKn8hXgEv1DXzL_zGWt4hiX_jTtE/copy. List of metabolites were paste into ‘IUPAC names’ column of above mentioned spread sheet and conversion was done following the script [=IMPORTDATA(“http://opsin.ch.cam.ac.uk/opsin/”&[SPREADSHEET CELL WITH IUPAC NAME]&“.stdinchikey”)] (Sitzmann et al., 2010; Lynch et al., 2020; Heller et al., 2015). Converted InChi keys were classified based on their kingdom, domain, class, subclass and parent level using web-based application ClassyFire accessible at <https://cfb.fiehnlab.ucdavis.edu/> (Djoumbou Feunang et al., 2016)

2.4. Volatile profiling of *Camellia sinensis*:

2.4.1. Plant-microbe Consortium Assembly and Cultivation Circumstances for Pot Experiments:

Soilrite mixture was subjected to autoclaved based steam sterilization. Upon sterilization pH was adjusted to 4.4 ± 0.2 . Surface sterilized seeds of *C. sinensis* were germinated on Soilrite mixture and kept inside the plant growth chamber (fitotron® SGC 120) maintaining temperature 28°C in the day and 22°C at night with a 12 hour light/dark cycle. Physiologically fit tea seedlings with equal number of leaves and equal height were considered for the pot experiment. Three sets of systems (T1, T2, and T5) were prepared containing 2 seedlings per set. T1 remains uninoculated with any microbial strain treated as control. Seedlings of the T2 set were inoculated only with *Trichoderma* sp. AM6 . Whereas, seedlings of the T5 set were inoculated with *Rhizobium* sp. DR23 (NCBI Accession: OR821747), *Bacillus* sp. D56 (NCBI Accession: OR821748), *Bacillus* sp. D42 (NCBI Accession: OR821750), *Bacillus* sp. DR15 (NCBI Accession: OR821749), 3g of crushed tea roots containing vesicular-arbuscular mycorrhiza (VAM) along with *Trichoderma* sp. AM6.

2.4.2. Development of plant VOCs trapping system:

A total of 3 of the 20-liter transparent water jars were sterilized with 70% ethanol. Sterilized inlet and outlet pipe were inserted by creating a hole within the jar. Each 5 ml pipet tip was filled with 500 mg Porapak Q 80/100 mesh (Sigma) and tightly packed with non-absorbent cotton to create a matrix as shown in [figure S1](#). Upon cutting edges of the jar, sets of plant systems were introduced aseptically within the jar and the bottom line of the jar was filled with sterile water. Cotton wick was introduced into the bottom line and extended to the surface of soil rites in the pot to facilitate capillary action, ensuring the plant receives a consistent water supply. All possible holes and cavities of the entire jar system were sealed with a Hillgrove electric 20W black mini hot glue gun. Emitted volatile organic compounds (VOCs) were collected from all experimental sets using the suction pump over a 5 minutes duration at each sampling point, across a total period of 21 days. During the entrapment process of VOCs, the air inlet was left open, while the outlet pipe was connected to an air suction pump. The entire VOCs trapping

system is schematically illustrated in [figure S1](#), providing a detailed overview of the setup and its components.

2.4.3. Quantification, Classification, and Comparative analysis of emitted VOCs using GC-MS :

The analysis of emitted, free and bound endogenous volatile compounds was conducted using a Thermo-Scientific GC-MS system (Trace 1300 GC equipped with ISQ QD, single quadrupole mass spectrometer). The GC was equipped with a split mode injector and a TG-5MS capillary column (length 30m, 0.32 mm i.d., 0.25µm film thickness). Injection volume of 1µl with a split ratio of 10:1 was maintained for GC analysis. The inlet temperature was set at 260°C. Compounds were separated with the following temperature method: initial oven temperature was set at 50°C for 2 min, followed by an increase of 60°C at a rate of 2°C min⁻¹ held for 2 min, then a linear increase of 3°C min⁻¹ to 210°C, held for 2 min followed by a final increase of 10°C min⁻¹ to 270°C and held for 7 min. Flow rate maintained for carrier gas helium was 1.5 ml min⁻¹. For mass spectrometer the following conditions were maintained: ion source temperature, 200°C; MS transfer line temperature, 280°C; electron energy, 70 eV and a scanning range of 40-600 a.m.u. Identification of volatile compounds was performed by comparing the spectral data with authentic standards and using the mass spectral library of NIST 14 (National Institute of Standards and Technology, Gaithersburg, MD). The compounds were confirmed by calculating the retention indices relative to C8-C40 n-alkanes. Quantification of volatile compounds was carried out using a known standard (ethyl hexanoate).

The entire range of VOCs emitted by all sets of *C. sinensis* was trapped and absorbed by Porapak Q 80/100 mesh (Sigma) as mentioned earlier. The process of eluting matrix-adsorbed volatile compounds involved the use of 200µL of HPLC-grade dichloromethane (DCM) in a glass vial. As an internal standard, 1µL of ethyl 6 bromo hexanoate (1:20 v/v in DCM) was added before injecting into the column.

Obtained peak areas were normalized concerning the internal standard. This process involved calculating the ratio of the peak area of each analyte to the peak area of the internal standard to achieve accurate quantification and account for any variability in sample injection and analysis conditions. A matrix file with the quantified values with the VOCs was generated and

subsequently uploaded to iMeta Lab sets explorer which is accessible at <https://shiny2.imetalab.ca/shiny/rstudio/SetExplorer/> to create an upset R and venn diagram for further analysis. The process of metabolite classification was the same as previously described in the LC-ESI-MS/MS section where metabolites were subjected to batch classification, followed by the conversion of InChI keys. Metaboanalyst 6.0 (<https://www.metaboanalyst.ca/>) was taken into consideration to generate a heatmap from normalized data of T1, T2, and T5 sets. The VOCs were hierarchically clustered based on their similarity across the three treatments.

2.5. Terpenoid Transcripts of *Camellia sinensis*:

Transcriptomics data sets from the NCBI Sequence Read Archive (SRA) under accession numbers SRS16999594 for 1T1, SRS16999596 for 3T2, and SRS16999595 for 2T5, were taken into consideration to obtain transcriptional responses of *C. sinensis* upon microbial inoculation (T2 and T5) and without any microbial inoculation (T1). Enzyme commission (EC) numbers assigned to genes were filtered from both upregulated and downregulated sets. Pathway mapping was performed using the KEGG Mapper tool (Kanehisa & Sato, 2020; Kanehisa et al., 2022).

3. Results:

3.1. DNA concentration, Purity and Quality:

Level of purity, quality and concentration of extracted genomic DNA are very crucial factors that influence the success of downstream sequencing applications. Extracted gDNA of *Trichoderma* sp. AM6 determined by nanodrop spectrophotometer (Thermo Fisher Scientific, USA) exhibited excellent purity with an A_{260}/A_{280} ratio of 1.88, indicating minimal contamination with proteins and other organics.

3.2. Genomic insights of *Trichoderma* sp. AM6:

3.2.1. Genome assembly:

Whole genome sequencing of *Trichoderma* sp. AM6 revealed 3,997,077 paired-end reads containing 603.5 Mbp total bases and 48% GC content. Box Whisker plot shown in [figure S2](#) representing ‘per base sequence quality’ revealed very high-quality calls as no base's lower quartile was less than 10 and no base's median was less than 25. Post-trimming sequence quality reanalysis shown in [figure S4](#) revealed no presence of any type of adapter content leading to pass in sequence quality control (QC). Genome assembly completeness analysis using BUSCO (Manni et al., 2021) shown in [figure XX](#) demonstrates that 98.8% of BUSCOs were classified as complete (C), with 1676 as single copy (S) and 10 as duplicate (D). Approximately 1% (17 BUSCOs) was found to be missing and a very small portion (0.1%) was classified as fragmented, indicating high levels of assembly completeness. The 39.91 Mbp genome assembly consists of 446 contigs in 406 scaffolds, of which 279 scaffolds are greater than 2 Kbp in length. A scaffold N50 value of 20 and L50 size of 0.60 Mbp indicates the level of assembly continuity as moderate. A total of 40 gaps accounts for only 0.01% of cumulative scaffolds length suggesting a low level of missing sequence data. [Table 1](#) shows the depth of assembled sequencing read coverage of *Trichoderma* sp. AM6 whole genome in tabular form.

3.2.2. Gene Ontology and KOG distribution analysis:

Gene ontology (Ashburner et al., 2000) analysis reveals the involvement of 4180, 2307, and 5384 gene counts in the Biological process (GO:0008150), Cellular component (GO:0005575), and Molecular function (GO:0003674) respectively. A total of 431 gene counts of which 364 are related to biological processes, 4 to cellular processes, and 63 to molecular functions were found to be obsolete. Entire gene ontology status of *Trichoderma* sp. AM6 is illustrated in [figure 1\(A\)](#). KOG (Clusters of Orthologous Groups) data represented in [figure 1\(B\)](#) categorizes genes based on Cellular Processes & Signaling, Information Storage & Processing, and Metabolism. However, genes with general predicted functions and unknown functions were kept within the poorly categorized category of KOG classification system. A total of 558 genes were found to be involved in posttranslational modifications, protein turnover, chaperones within cellular processes & signaling categories. Signal transduction mechanisms are prominent with 424 genes followed by Intracellular trafficking, secretion, and vesicular

transport with 301 genes. Comparatively less prominent functions, like cell motility, were found to be only 5 genes. The information storage and processing class emphasizes RNA processing and modification (236), Chromatin structure and dynamics (89), Translation, ribosomal structure and biogenesis (350), Transcription (278), Replication, recombination, and repair (200) functions. The Metabolism category is dominated by genes related to energy production containing 448 genes and lipid metabolism containing 394 genes. A significant portion belonging to the poorly characterized category is predicted to have 1311 genes with general functions and 368 with unknown functions.

3.2.3. Transporters & Transcription factors:

A total of 1073 transporters predicted using TCDB (<https://tcdb.org>) were found to be present in *Trichoderma* sp. AM6 genome. Among them, 554 was annotated as electrochemical potential-driven transporters which utilize electrochemical gradients for the movement of substances across cell membranes. Uniporters, symporters, and antiporters belong to this category. 248 annotations were related to primary active transporters that rely upon direct energy sources like ATP. Although 3 vesicle fusion pores were present along with 57 α -type channels and 4 β -barrel porins, no viral fusion pore was found to be present. However, a total of 97 belonged to incompletely characterized transport systems.

Fungal Zn(2)-Cys(6) binuclear cluster domain (PF00172) was found to be most abundant followed by fungal specific transcription factor domain (PF04082, PF11951), Acetyltransferase (GNAT) family (PF00583), SET domain (PF00856). Different types of zinc finger domains including C2H2, C3HC4 (ring finger), ZZ, C5HC2, MIZ/SP-RING, and GATA were identified in the annotation. However, other transcription factors include Homeodomain (PF00046), bZIP (PF00170), SNF2 domain (PF00176), TFIID or TBP (TATA-binding protein), WW domain (PF00397), HMG (high mobility group) box, DDT domain (PF02791), TEA/ATTS domain (PF01285) etc. Detailed annotation of transporters and transcription factors of *Trichoderma* sp. AM6 has been provided in [supplementary Excel files X1.xls and X2.xls](#) respectively.

3.2.4. Metabolic Pathway Analysis:

Kyoto Encyclopedia of Genes and Genomes (KEGG) was used to link genomic data with systematic information on metabolic pathways (Kanehisa & Goto, 2000). Overview of KEGG metabolic pathways of *Trichoderma* sp. AM6 has been depicted as a sunburst plot in [figure 1\(C\)](#). Among all, the number of enzymes predicted in *Trichoderma* sp. AM6 involved carbohydrate metabolism pathways (385) was found to be most abundant followed by amino acid metabolism (348). In addition, a total of 89 were involved in the biosynthesis of secondary metabolites as represented in the bar diagram in [figure 1\(D\)](#). Among them, 12 enzymes were especially involved in terpenoid backbone biosynthesis. Table 2 provides detailed information on these enzymes, including the protein name, protein ID, E-value, top KEGG hit, EC number, and the corresponding enzyme names along with their curation status. Terpenoids, popularly known as isoprenoids, belong to a highly diverse group of natural products consisting of isoprene (C5) units (Niu et al., 2017). The Mevalonate pathway [MD:M00095] and MEP/DOXP pathway [MD:M00096] are two possible biosynthetic pathways for terpenoid building blocks. However, the non-mevalonate pathway, so called MEP/DOXP pathway is generally absent in lower eukaryotes but in plants they are found to be coexisting together in separate cellular compartments (Lange et al., 2000). Terpenoid backbone biosynthesis (MAP00900) mapping of *Trichoderma* sp. AM6 genome represented in [figure 2](#) revealed the involvement of the mevalonate pathway and the absence of MEP/DOXP pathway. Acetyl Co-A from glycolysis results in isopentyl-PP through several intermediates including acetoacetyl Co-A, mevalonate, mevalonate-5P and mevalonate-5PP. *Trichoderma* sp. AM6 genome possesses the ability to further convert isopentyl-PP into dimethylallyl-PP (precursor of zeatin biosynthesis), geranyl-PP (precursor for monoterpenoid biosynthesis), and geranyl geranyl-PP (precursor for diterpenoid biosynthesis) as shown in the metabolic map of [figure 2](#).

3.2.5. Secondary metabolite gene clusters:

Trichoderma sp. AM6 whole genome mining revealed a diverse range of biosynthetic gene clusters (BGCs) upon analysis by fungiSMASH (Blin et al., 2023). The genome harbors a wide range of BGCs types including Terpene, Type 1 Polyketide synthase (T1PKS), Non-ribosomal peptide synthase (NRPS), NRPS-like, isocyanide-NRP, fungal-RiPP-like clusters with similarity to known metabolite ranging from 10% to 100% as shown in [Table 2](#). The T1PKS cluster was found to be the most abundant with several 17 whereas a single isocyanide-NRP

cluster was present. Both terpene and NRPS clusters were represented by 7 clusters each. A total of 12 hybrid clusters, defined as clusters containing more than one biosynthetic type, were also identified. Several regions revealed 100% similarity to known metabolites such as clavatic acid (Region 22.2), peramine (Region 333.4), harzophilone (Region 351.3) and choline (Region 382.1). Moderate similarities ranging from 55-80% were observed within the region linked to several metabolites such as harzianopyridone (Region 373.2), metachelin (Region 144.2), trichoxide (Region 155.1), dichlorodiaporthin (122.1), tricholignan A (299.1), trichobrasilenol (Region 356.3). However, regions with lower similarities were also found to be present indicating the potential for novel compounds, with decumbenone (Region 351.1, 15%), leucinostatin (Region 333.2, 10%), aurofusarin (Region 122.2, 18%). A substantial amount of the biosynthetic gene clusters (BGCs) identified from *Trichoderma* sp. AM6 whole genome assembly exhibited no significant similarities with any previously defined clusters as mentioned in [Table ST1](#).

3.2.6. Comparative Genomics and Phylogeny Analysis:

Orthologous analysis, phylogenetic analysis and expansions-contractions analysis were carried out using OrthoVenn3 (Sun et al., 2023). Species-specific functional diversity has been depicted in the occurrence plot shown in [figure 3](#). A total of 7,296 gene clusters are shared across 8 selected *Trichoderma* strains. *Trichoderma* sp. AM6 exhibits the highest number of proteins (69,569), largest gene clusters (15,013) and highest count of singletons (8,207) compared to others. Notably 2,260 gene clusters with 6,852 protein counts are exclusively unique to *Trichoderma* sp. AM6. [Table 3](#) highlights the total number of proteins, gene clusters, and singletons across selected *Trichoderma* strains. Evolutionary relationships among them have been reconstructed using the maximum-likelihood approach ([figure S5](#)). The amino acid substitution model (JTT) combined with a specific site rate variation model (CAT) was taken into consideration for variability in evolutionary rates across sites (Wang et al., 2008). Species tree revealed numerous distinct clades indicating evolutionary divergence within *Trichoderma* species. *Trichoderma* sp. AM6 was found in the same clade as *Trichoderma simmonsii* GH-Sj1 and *Trichoderma harzianum* suggesting their close evolutionary relationship. The ultrametric tree in [figure 4](#) reveals gene family expansion and contraction patterns. Expansion and contraction (+1/-5) shown at the root of the tree indicate that the size of the gene family

featured relatively few changes in the earliest progenitors with more dramatic evolutionary changes in the lineage. *Trichoderma* sp. AM6 exhibits highest expansion of gene families with less contraction whereas, *T. reesei* shows the highest number of gene family contractions with minimal expansion. Branch node leading to *T. simmonsii* GH-Sj1, *T. sp.* AM6, *T. harzianum* and *T. semiorbis* FJ059 shows a large expansion (+668) with only a few (-9) gene families contraction, implying that these species might share common adaptive traits that stem from their shared ancestor.

3.3. Metabolomic insights of *Trichoderma* sp. AM6:

Untargeted LC-MS identified the presence of a total of 11,841 metabolites from the crude extract of 12 days old *Trichoderma* sp. AM6 culture. Upon converting those metabolites into InChI key, no key was found for 1218 compounds indicating possible unknown or novel metabolites within the dataset. The remaining 10,623 compounds were subjected to ClassyFire batch classification where 10,013 belong to the ‘organic compound’ class, 7 belong to the ‘inorganic compound’ class and 603 remain unclassified at the Kingdom level. Superclass level classification revealed organoheterocyclic compounds (4,496) to be most abundant followed by benzenoids (2,446). However, lipid and lipid-like molecules consisted of 497 metabolites which were further divided into total 6 classes (Fatty acyls, Prenol lipids, Steroids and steroid derivatives, Glycerolipids, Glycerophospholipids and Saccharolipids). Our molecule of interest terpenoid (monoterpenoid and diterpenoid) belongs to the ‘Prenol lipids’ class. Among 268 prenol lipid classes, 72 diterpenoid and 34 monoterpenoid compounds (metabolites) were present as shown in the doughnut chart in [figure 5](#). Detailed level-specific (including kingdom, superclass, class, subclass and parent level) classification of *Trichoderma* sp. AM6 has been represented in pie charts in [figure S6](#). Supplementary 2.xls contains names of all metabolites, InChI key and the entire classification system.

3.4. Volatile profiling of *Camellia sinensis*:

The total ion chromatogram (TIC) represented in [figure S7](#) summed up the intensities across the entire range of masses being detected at every point in the analysis. However, TIC shown in

figure S8 represents the relative abundance of T1, T2 and T5 peak sets with respect to retention time (minute) in a comparative fashion.

3.4.1. Quantification of volatile organic compounds (VOCs):

Ethyl 6 bromohexanoate ($C_8H_{15}BrO_2$) and dichloromethane (DCM) were mixed with 1:20 ratio. Molecular weight (M_w) of Ethyl 6 bromohexanoate is 223.106 g/mol and density is 1.254 g/mL at 25°C. 21 μ L of solution was prepared (1 μ L ethyl 6 bromohexanoate + 20 μ L DCM from 200 μ L elution volume). From the entire homogenized solution 1 μ L was injected to the GC sample inlet. Concentration of internal standard has been calculated as follows:

$$\text{Mass (g)} = \text{volume (L)} \times \text{Density (g/mL)} = 1 \times 10^{-6} \times 1.254 \text{ g}$$

$$\text{Concentration (M)} = [\text{Mass of solute (g)}/\text{Volume of solution (L)}] \times [1/M_w]$$

$$= (1.254 \times 10^{-6} \times 1)/(21 \times 10^{-6} \times 223.106) \text{ [as, total volume of solution} = 21 \mu\text{L} = 21 \times 10^{-6} \text{ L]}$$

$$= 2.67 \times 10^{-4} \text{ mol/L or M}$$

Now, 1 mol of ethyl 6 bromohexanoate is equivalent to 223.106 g

$$\text{So, Concentration of internal standard (IS)} = \text{molarity (M)} \times \text{molar mass (g/mol)}$$

$$= 2.67 \times 10^{-4} \times 223.106$$

$$= 0.059569 \text{ g/L}$$

$$= 59.569 \text{ mg/mL}$$

Normalized peak area of ethyl 6 bromohexanoate was 166695043.494022 which corresponds to the concentration 59.569 mg/mL. Based on the concentration and peak area of the internal standard (ethyl 6 bromohexanoate), concentration of all identified VOCs were calculated.

Table ST2 represents all quantified values of VOCs across T1, T2 and T5 experiment sets.

3.4.2. Comparative analysis of VOCs across T1, T2 and T5 sets:

Distribution pattern of *C. sinensis* volatile profiles across control (T1), only *Trichoderma* sp. AM6 treated (T2) and *Trichoderma* sp. AM6 with other microbial consortia-treated experimental sets has been depicted in figure 6(A). The venn-euler diagram reveals only 41 metabolites (VOCs) are common within the above-mentioned experimental sets. Internal standard inflates one unit count across the overlapping regions. The heat map generated from

the 40 common VOCs using MetaboAnalyst 6.0 has been represented in [figure 6\(C\)](#). Specific patterns of heatmap reveal T1 as the highest abundance compared to others, whereas T2 exhibits a moderate level of abundance. T5 sets showing the lowest level of VOCs abundance have several compounds with high concentrations including paromomycin, 2-fluoro- β ,3-dihydroxy-N-methyl-benzeneethanamine, 1,3-dimethyl-benzene and moderate levels of concentrations like argon, cystine. However, T1 and T2 sets share a total of 59 (41+20-2) common metabolites. Heatmap of VOCs emission variation between T1 and T2 sets are represented in [figure 6\(D\)](#). A total of 10 among them are abundant in T2 sets. These VOCs comprise paromomycin, cystine, argon, propane, propyne, 1-ethyl-3-(1-methylethyl)-benzene, N-carbobenzyloxy- β -alanine, 2-Furanmethanol, 1-(4-ethylphenyl)-ethanone and tert-butyl-oxyformamide. T1 contains 49 unique VOCs, T2 includes 47 unique VOCs, while T5 set comprises 34 unique VOCs indicating treatment specific volatile compounds. Detailed classification of total 205 volatile organic compounds released by *C. sinensis* plant under control and experimental setups along with internal standard are provided in [supplementary excel file X](#). As previously mentioned, terpenoid backbone leading to the formation of compounds like monoterpenoids, diterpenoids, tetraterpenoids, and sesquiterpenoids falls under ‘prenol lipid’ class category of ‘lipid and lipid-like molecule’ superclass of ‘organic compounds’ kingdom in the chemical classification system used in our study.

3.5. Transcriptomic insights of *Camellia sinensis*:

Overview of secondary metabolite biosynthesis metabolic map constructed using differentially expressed datasets of T1 vs T2 experimental as depicted in [figure S9](#). Terpenoid biosynthesis pathway highlighting different regulation of mevalonate pathway (cytosolic) as well as non mevalonate or 1-deoxy-d-xylulose-5-phosphate (DOXP) / 2C-methyl-d-erythritol-4-phosphate (MEP) pathway (MEP/DOXP) pathway (plastidial) has been represented in [figure 7](#). The mevalonate pathway that begins in plant cytoplasm with acetyl-CoA is responsible for the production of mevalonate, an intermediate of several terpenoid compounds like sesquiterpenoids, triterpenoids. 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), a key regulatory enzyme in this pathway, converts HMG co-A to mevalonate. On the other hand MEP/DOXP pathway, occurring in plant plastids, initiates from pyruvate and leads to formation of monoterpenes, diterpenes, isoprenes through the production of notable

intermediates like 1-Deoxy-D-xylulose 5-phosphate (DXP), 2-C-Methyl-D-erythritol 4-phosphate (MEP), Dimethylallyl-PP (DMAPP), geranyl-PP (GGPP), geranyl geranyl-PP (GGPP). Upregulated transcripts of T1 vs T2 sets exhibit presence of HMGCR (E.C.1.1.1.34) whereas the downregulated transcripts of T1 vs T2 sets reveal presence of DXS (E.C. 2.2.1.7) which catalyzes the formation of 1-Deoxy-D-xylulose 5-phosphate (DXP) from pyruvate. Interestingly, upregulated transcripts of the T1 vs T5 set revealed the presence of HMGCR. However, no key enzymes involved in the terpenoid biosynthesis pathway were found to be present in the downregulated transcripts of T1 vs T5 sets.

Discussion:

Camellia sinensis L., commonly called tea plant, is a dicotyledonous perennial commercial crop that belongs to Theaceae family. According to Lanjekar, 2022 billions of hot or cold cups of tea brewed from a range of cultivars and types, including *Assamica* (large leaf), *Cambodi* (middle leaf), and *Sinensis* (small leaf), are consumed daily by people worldwide. More than 85% of the world's tea requirement is fulfilled by several Asia-Pacific nations among which China, India, and Sri Lanka are top (Rathnayake et al., 2021). Due to continuous climate change of those nations producers encounter numerous challenges hidden beneath the serene beauty of the tea gardens. Apart from several abiotic stresses, resilient yet vulnerable tea plants constantly suffer from several insidious diseases like brown blight, leaf miner, algal leaf spot, root rots, cankers etc. (Soeb et al., 2023; Pandey et al., 2021). According to Chen & Chen, 1990 around 507 fungal pathogens are associated with tea plants. Climate-fungal pathogen modeling by Tibpromma et al., 2021 predicted up to one-third loss of tea growing areas. Global transcriptome analysis by Hazra et al., 2023 also revealed fungal infections as an inevitable limiting factor for productivity of tea. Since 1920, a wide group of filamentous fungi typically found in soil, plants and wood belonging to the genus *Trichoderma* is very well known for their ability to function as biocontrol agents against several plant pathogens. *Trichoderma* was formerly believed to act upon pathogens by mycoparasitism, antibiosis, and competition (Harman, 2006). However, recent studies demonstrate their abilities to colonize root epidermis and outer cortical layer triggering local and systemic resistance followed by releasing of several

601 bioactive molecules that alter transcriptome and proteome of host plants (Sharma et al., 2017;
 602 Dutta et al., 2023). Sood et al., 2020 described *Trichoderma* as a ‘Multitalented Biocontrol
 603 Agent’. Nonetheless, in recent times *Trichoderma* spp. are proposed by researchers as major
 604 plant growth-promoting fungi (PGPF) with the ability to boost plant growth and productivity.
 605 Rush et al., 2021 describes *Trichoderma* spp. as a valuable source of natural products.
 606 Schalamun & Schmoll, 2022 pointed out *Trichoderma* genome and genomics as treasure troves
 607 towards biological, biotechnological and agricultural research. Our study reveals the potential
 608 of *Trichoderma* sp. AM6 through whole-genome analysis, extending beyond its conventional
 609 biocontrol properties.

610 Over 500 of *Trichoderma* species have been reported and identified globally (Sousa et al.,
 611 2023; Ye et al., 2023;). The majority of studies reported rhizospheric soil as the source of
 612 *Trichoderma* isolation. However, *Trichoderma* sp. AM6, the strain used in our present study
 613 was isolated from surface sterile roots of *Camellia sinensis*. As of right now, the NCBI database
 614 contains only 20 complete assembly-level genomes of *Trichoderma*, while the JGI MycoCosm
 615 database has a total of 88 publicly available genomes. The JGI Annotation pipeline was
 616 utilized to annotate the *Trichoderma* sp. AM6 whole genome following quality control,
 617 trimming, and assembly of raw reads. The genomic insights of this particular *Trichoderma*
 618 strain revealed its ability to synthesize terpenoid backbone (figure 1) which could result in
 619 synthesis of several mono, di, tri and sesquiterpenoids along with steroid, carotenoid, zeatin and
 620 indole diterpene alkaloid as depicted in figure 2. Terpenoids have been proven to have a variety
 621 of biological actions, in addition to contributing significantly to the floral odors of plants (Singh
 622 & Sharma, 2014). Zeng et al., 2017 discussed the role of terpene volatiles in tea plants during
 623 plant to plant communication. Several sesquiterpene compounds also act as signal volatile
 624 resulting in biotic and abiotic stress responses also. Terpenoids and steroid derivatives
 625 particularly derived from natural origins are of great interest due to their antidiabetic property,
 626 as they lower blood glucose by regulating glucose metabolism, prompting diverse synthetic
 627 efforts to modify the terpenoid structure with various chemical groups (Ghani, 2020; Shehadeh
 628 et al., 2021). Moreover, terpenoids also have anti-inflammatory, anti-malarial, anti-cancer, and
 629 anti-ulcer properties in addition to their antioxidant benefits (Adefegha et al., 2022). Terpenoids
 630 have been found to possess therapeutic potential against protozoan parasitic diseases based on
 631 empirical evidence from a number of bioassays (Isha et al., 2018). Consumption of tea, on the
 632 other hand, has been used for ages to strengthen the immune system and fight off combat

633 diseases. Conducted research investigations, meta analysis and epidemiological studies
 634 acknowledged substantial health-promoting properties of tea including antioxidative, anti-
 635 inflammatory, antimicrobial, anticarcinogenic, antihypertensive, neuroprotective, cholesterol-
 636 lowering properties with associate disease prevention abilities such as cancer, diabetes,
 637 arthritis, cardiovascular disease (CVD), stroke, genital warts, and obesity etc. (Hayat et al.,
 638 2015; Pak et al., 2024; Yang et al., 2018; Sagris et al., 2024). So, consumption of tea with added
 639 terpenoids may synergistically support glucose metabolism, reduce oxidative stress, and
 640 improve cardiovascular and immune health that for sure will enhance its therapeutic potential,
 641 amplifying tea's role in disease prevention and health promotion.

642 Though plenty of focus has been on terpenoids produced by plants, some fungi also have the
 643 ability to synthesize terpenoids. Till now there are two well established primary mechanisms
 644 involved in synthesis of terpenoids are mevalonate and non-mevalonate. Interestingly the non
 645 mevalonate pathway which is also referred to as MEP/DOXP pathway is absent in higher
 646 eukaryotes as well as in fungi (Zhao et al., 2013). Pathway analysis of *Trichoderma* sp. AM6
 647 whole genome performed in this study and shown in [figure 2](#) revealing the involvement in
 648 mevalonate pathway instead of non mevalonate pathway supports the above statement.
 649 However, higher eukaryotes, especially green plants use both non mevalonate as well as
 650 mevalonate simultaneously in separate cellular compartments. Significant increase in the
 651 terpenoid level of *Camellia sinensis* will undoubtedly amplify its multifaceted benefits, ranging
 652 from bolstering plant defense mechanisms to improving human health through dietary
 653 consumption. With an eye toward triterpenoid engineering, the least engineered class of
 654 bioactive terpenoids, Moses et al., 2013 explored a number of synthetic biology techniques to
 655 improve and diversify the synthesis of plant terpenoids. In addition to mentioning endophytes
 656 as a novel source of plant terpenoids Chen et al., 2021 also discussed their ability to produce
 657 terpenoids by themselves along with stimulating the terpenoid accumulation in their host
 658 plants. One of the major questions our study seeks to address is: To what extent does the
 659 endophytic nature of *Trichoderma* sp. AM6 enhances terpenoid content in *C. sinensis*, the host
 660 plant, either through direct synthesis of terpenoids or by stimulating the host's terpenoid
 661 biosynthetic pathways? Untargeted LC-ESI-MS/MS analysis was carried out to detect the
 662 terpenoids produced by *Trichoderma* sp. AM6 which revealed the presence of 182 types of
 663 terpenoids from mentioned fungal extract as shown in [figure 5](#). The combination of genomic
 664 predictions along with metabolomic validation from our study provides strong evidence

665 towards the capability of *Trichoderma* sp. AM6 to produce terpenoids, highlighting its
 666 potential role as a direct source. Li et al., 2023 highlighted the role of pot level experiments and
 667 controlled set ups for investigating the modulation of several biosynthetic pathways. Pot level
 668 experiments were performed in our study using two particular plant-microbe consortium
 669 assemblies as mentioned in the material and method section. Recently, extensive bacterial
 670 genome sequencing and bioinformatic analysis of deduced bacterial proteins using a profile
 671 hidden Markov model have revealed more than a hundred distinct predicted terpene synthase
 672 genes (Yamada et al., 2013). This study also investigates how the presence of tea root-
 673 associated bacteria, in combination with *Trichoderma* sp. AM6 inoculation influences the
 674 regulation of the terpenoid synthesis pathway in tea plants. The pot level experiments included
 675 a control set (T1) without microbial inoculation, a set inoculated with only *Trichoderma* sp.
 676 AM6 (T2), and a set treated with *Trichoderma* sp. AM6 and other tea root associated microbes
 677 (T5). The RNA-seq data from different experimental conditions of microbial inoculation
 678 provides intriguing insights into the regulation of key enzymes in the terpenoid biosynthesis
 679 pathways. Upregulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) in
 680 both the T2 and T5 sets suggests that these conditions might favor the mevalonate pathway for
 681 terpenoid synthesis instead of non mevalonate pathway. HMGCR, being a rate controlling
 682 enzyme, plays a critical role in the production of mevalonate from 3-hydroxy-3-methylglutaryl
 683 coenzyme A (HMG coA), a precursor for downstream terpenoid compounds like
 684 sesquiterpenes, sterols, triterpenoids. This upregulation upon *Trichoderma* sp. AM6
 685 inoculation clearly indicates a metabolic shift towards enhanced synthesis of terpenoids in *C.*
 686 *sinensis* cytosol as mentioned using green and purple arrow in [figure 6](#). On the other hand,
 687 downregulation of 1-deoxy-D-xylulose-5-phosphate synthase (DXS) in the non-mevalonate
 688 pathway in T2 but not in T5 implies differential regulation of terpenoid biosynthesis pathway,
 689 which is crucial for the production of particularly mono and diterpenoids. The absence of DXS
 690 downregulation in the T5 set raises the possibility of a delicate balancing act between the two
 691 pathways. A clearcut metabolic crosstalk in tea plants between the mevalonate and non-
 692 mevalonate (MEP/DOXP) pathways upon microbial inoculation is observed in our study.
 693 Therefore, our study concludes that the endophytic nature of *Trichoderma* sp. AM6 increases
 694 the amount of terpenoid content in *C. sinensis* by both directly synthesizing terpenoids as well
 695 as indirectly inducing the host's terpenoid biosynthesis pathways. To better understand the

functional effects of this regulation, further studies on the downstream metabolites generated under T2 and T5 circumstances was necessary.

An easy and cost effective plant volatile trapping system was constructed as shown in [figure S1](#) that enabled the efficient collection of volatile organic compounds (VOCs) and an untargeted GC-MS analysis method was employed to analyze the specialized metabolites from T1, T2 and T5 sets. Volatiles are essential quality ingredients of tea plant or *Camellia sinensis*. Plant development can be enhanced by microbial volatile organic compounds (VOCs) that control photosynthesis, nutrient uptake, phytohormone activity, and metabolic processes as well in intra and inter-kingdom interactions (Sun et al., 2020; Martín-Sánchez et al., 2020; Schulz-Bohm, 2017). Plants emit diverse volatile organic compounds (VOCs) in response to biotic and abiotic stresses, acting as infochemicals in the phytobiome. In addition to promoting the growth of tea plants, colonized microorganisms have an impact on the synthesis of volatile organic compounds (VOCs), which are essential for plant defense and quality (Tang et al., 2024; Sharifi et al., 2018). The core volatile chemicals, which are consistently produced regardless of inoculation may be crucial elements of the tea plant's main metabolism or baseline defensive mechanisms, were represented by the 40 VOCs that were prevalent in all three circumstances. The combination of *Trichoderma* sp. AM6 and other tea root-associated microbes led to the emission of fewer VOCs than the other treatments suggesting that the combined inoculation might suppress certain volatile pathways while enhancing others, reflecting a potentially synergistic or competitive interaction between the microbes that influences the plant's metabolism. *Trichoderma* is well known for priming plant defense mechanisms, as was previously indicated. The unique volatiles observed in the *Trichoderma* sp. AM6 inoculated set (T2) suggests activation of specific defense pathways, potentially enhancing the plant's systemic resistance against pathogens. The distinct changes in VOC profiles in T2 underscore the ability of *Trichoderma* sp. AM6 to modulate plant metabolism, equipping tea plants to better tolerate environmental stresses. By inducing metabolic shifts, *Trichoderma* not only enhances plant fitness but also fortifies its capacity to withstand microbial stress. Quantification of volatiles using GC-MS revealed variations in volatile concentrations across treatments, highlighting the influence of *Trichoderma* sp. AM6 inoculation on *C. sinensis* metabolism. However, the source of the unique number of volatiles from T2 (47) and T5 (34) sets are not clear as they could be either microbial volatiles (mVOCs) or microbe induced plant volatiles (MIPVs). Further investigation using axenic cultures of microbes and dual-chamber systems

could isolate mVOCs, while inoculation with heat-killed microbes or microbial exudates can help identify MIPVs. Distinguishing these sources and understanding their roles in systemic resistance and plant-microbe interactions will offer valuable insights into microbial contributions towards metabolic profiling of tea plants.

The genomic landscape of *Trichoderma* sp. AM6 serves as a treasure trove of multifunctional potential, unveiling diverse capabilities for plant-microbe as well as microbe-microbe interaction. The final question we sought to answer is how the strain in our study, *Trichoderma* sp. AM6 differs from other well known *Trichoderma* species in terms of genomic features, ecological adaptability, evolutionary insights and functional potential? Comparative genomics study helps us to uncover evolutionary relationships along with similarities and differences between multiple *Trichoderma* species as mentioned earlier. Substantial genomic changes, particularly huge acquisition of genes with a nominal number of loss makes *Trichoderma* sp. AM6 is very unique among all *Trichoderma* species. Its significant divergence, in contrast to other *Trichoderma* species, emphasizes its evolutionary distinctiveness and highlights its significance for further study. This could have implications in industrial as well as agricultural applications, such as enhanced production of terpenoids, flavonoids along with other valuable secondary metabolites. Extensive whole-genome, transcriptome and metabolome analyses along with integrated pathway analysis of *Trichoderma* sp. AM6 performed in our study provides valuable insights into its genetic and biochemical landscape, invites deeper exploration into its exceptional potential as a model organism for studying large-scale gene acquisition events and their transformative impact on phenotypic traits, particularly in the context of plant-microbe interactions.

Data availability:

Trichoderma sp. AM6 genome sequence, assembly and annotations are available from JGI MycoCosm (https://mycocosm.jgi.doe.gov/TrispAM6_1) and have been deposited to NCBI databanks WGS: SUB13495275; BioProject: PRJNA980546; BioSample accession: SAMN35637920; Genbank accession: JAUJDX0000000000

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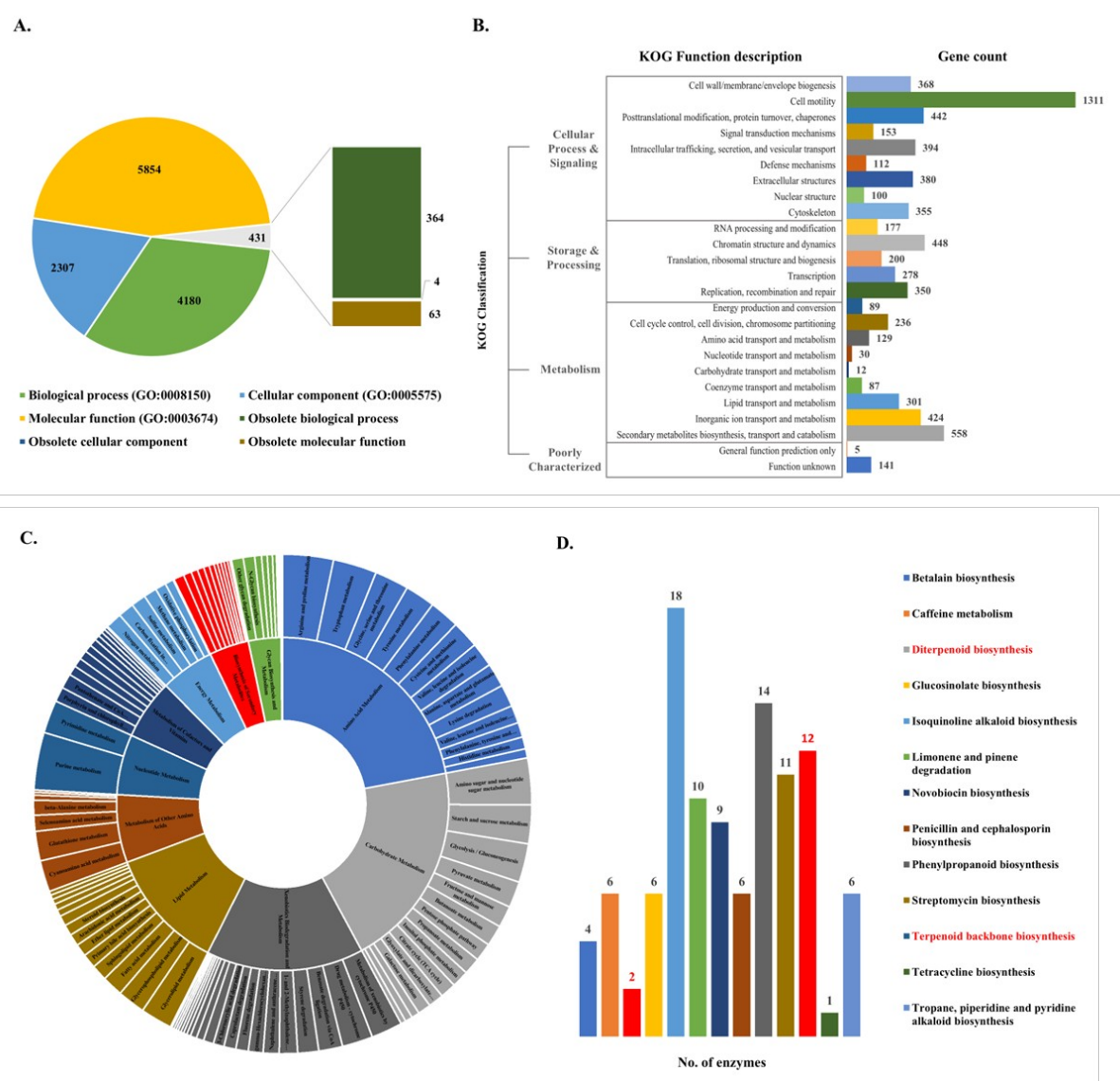


Figure 1: Overview of *Trichoderma* sp. AM6 whole genome annotation. (A) Gene ontology (GO) status represented as pie diagram. Bar of pie indicates obsolete functions. Numbers inside the box specify the involvement of genes across different categories. (B) Summary of Eukaryotic Orthologous Groups (KOG) distribution pattern. (C) Sunburst plot illustrating KEGG pathway enrichment analysis of metabolic processes across 10 categories including carbohydrate, lipid, nucleotide, amino acid, xenobiotics, glycan, vitamins and co-factors

metabolism etc. which makes up the first circle inside. Outer circle represents subcategories among those primary categories. ‘Biosynthesis of secondary metabolism’ category highlighted in red color reflects specific secondary metabolite biosynthesis relevant to our study. (D) The bar chart representing the number of enzymes present within *T. sp.* AM6 genome and their involvement contributing several biosynthesis pathways. Terpenoids being molecules of interest enzymes involved in ‘Diterpenoid biosynthesis’ and ‘Terpenoid backbone synthesis’ pathways are highlighted in red.

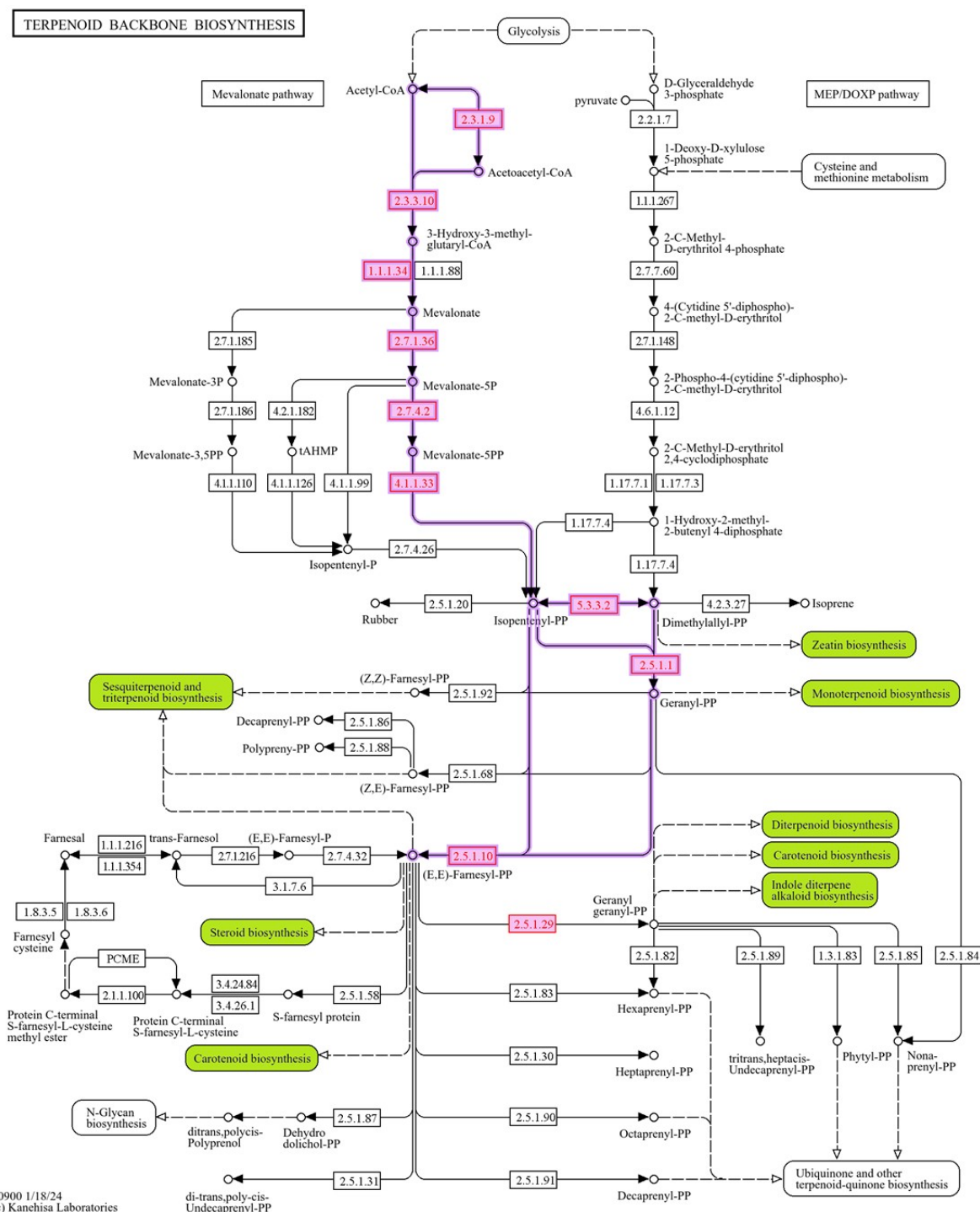


Figure 2: KEGG pathway map of terpenoid backbone biosynthesis (MAP00900). Mappable enzymes of this pathway present within *Trichoderma* sp. AM6 genome are highlighted in pink.

Violet line represents the network connection. Green boxes indicate possible types of metabolites from the genome. Detailed description of enzyme commission numbers (inside the box) has been provided in table 3.

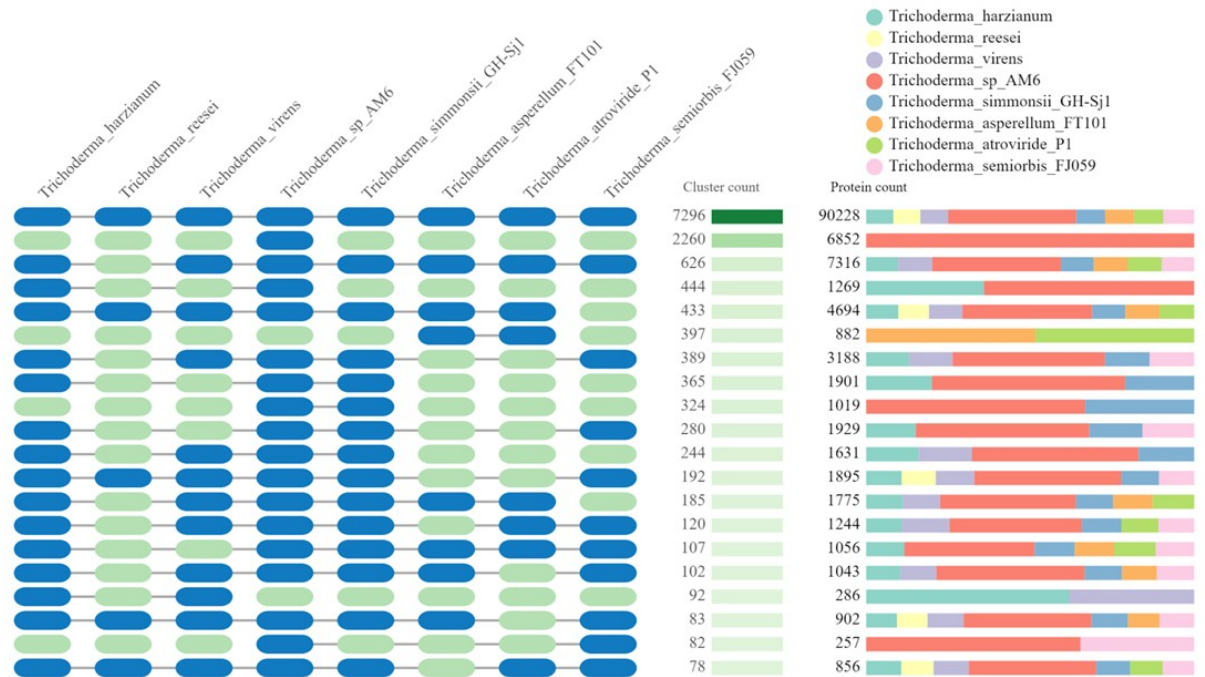


Figure 3: Occurrence plot illustrating species-specific functional diversity across 8 selected *Trichoderma* species. The colored bars illustrate the presence and absence of clusters. Protein counts associated with each cluster are also represented in bar diagrams with specific color codes for specific strains of *Trichoderma*.

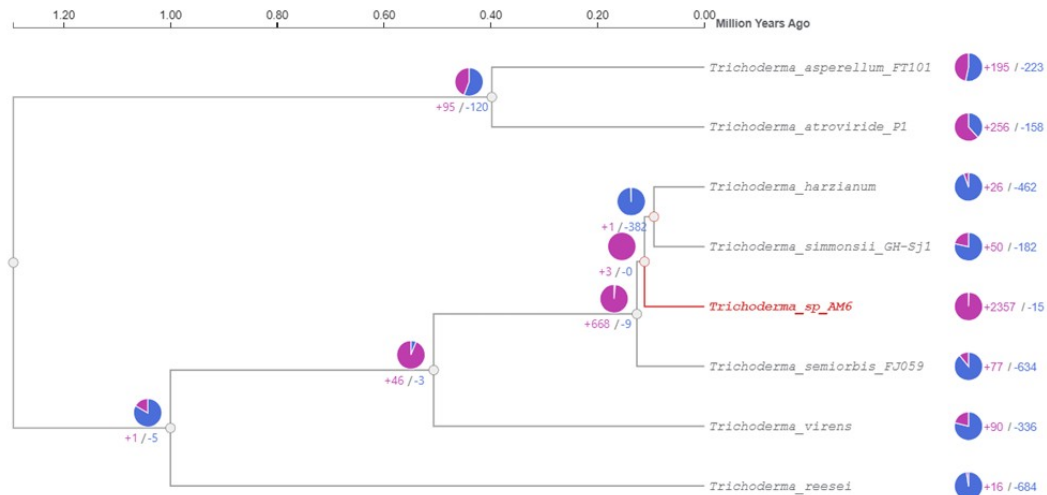


Figure 4: Ultrametric tree representing expansion and contraction analysis of gene families across selected *Trichoderma* strains. Scale at the top indicates branch length as the number of millions of years ago (MYA). Gene family expansion is decoded by violet while contraction is decoded by blue with corresponding numerical values indicating the number of expanded (+) or

contracted (-) gene families. *Trichoderma* sp. AM6 being the strain of interest is highlighted in red.

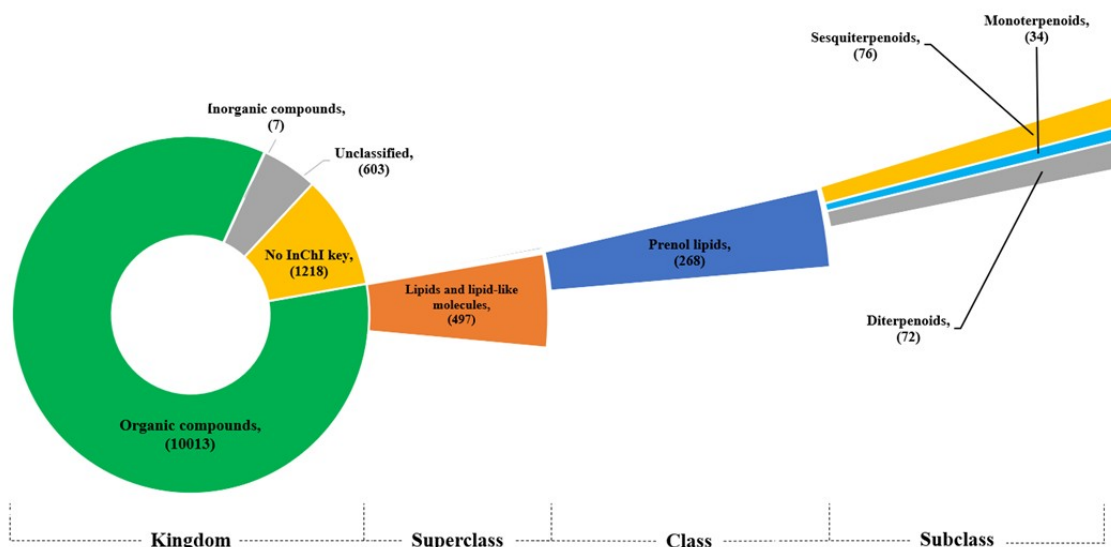


Figure 5: Representation of targeted analysis from untargeted metabolomics (LC-MS). Among the total of 11841 detected secondary metabolites from *Trichoderma* sp. AM6 from across multiple levels of chemical taxonomy including kingdom, superclass, class and subclass from untargeted 34 monoterpenoids, 72 diterpenoids and 76 sesquiterpenoids were revealed.

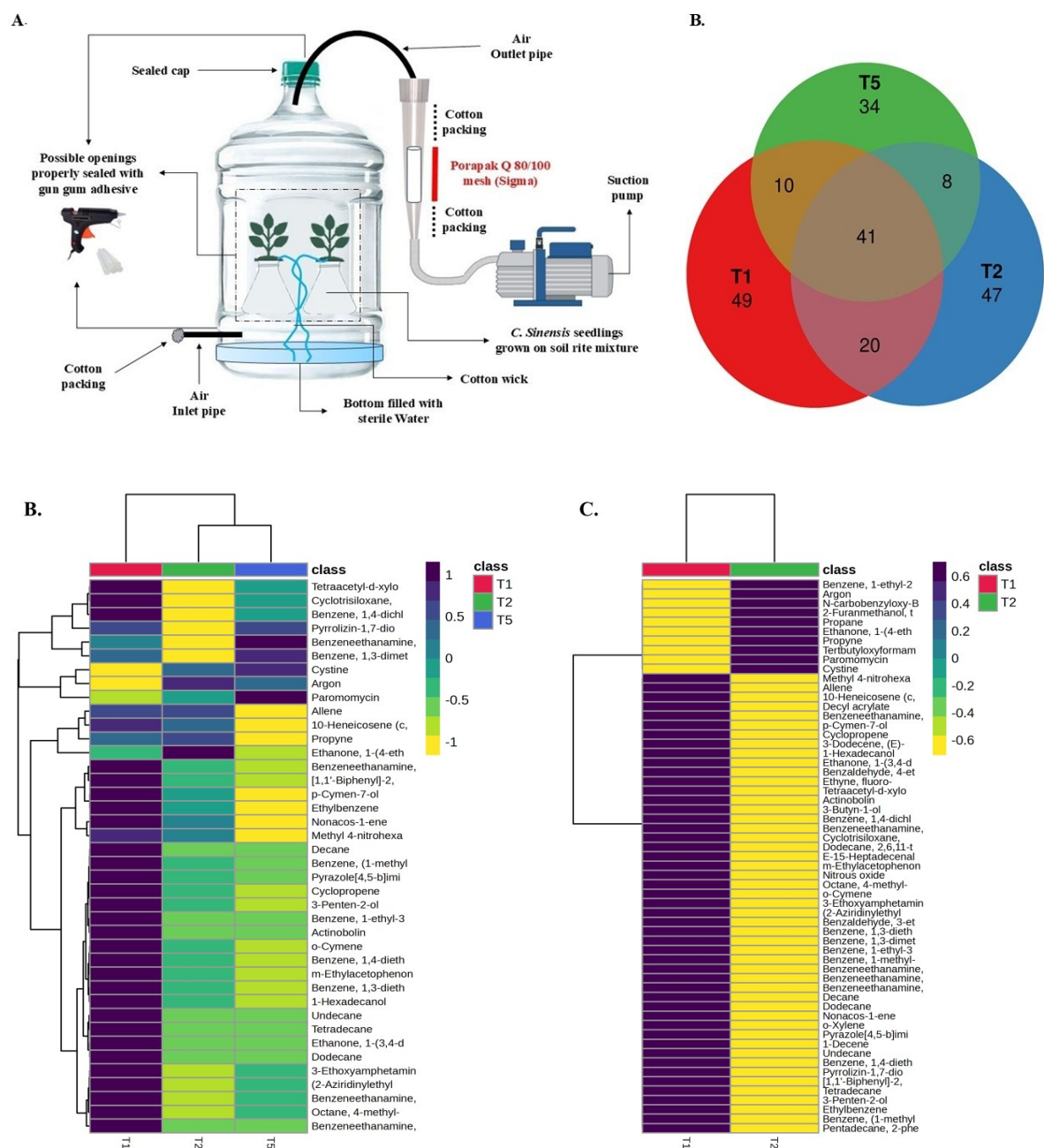
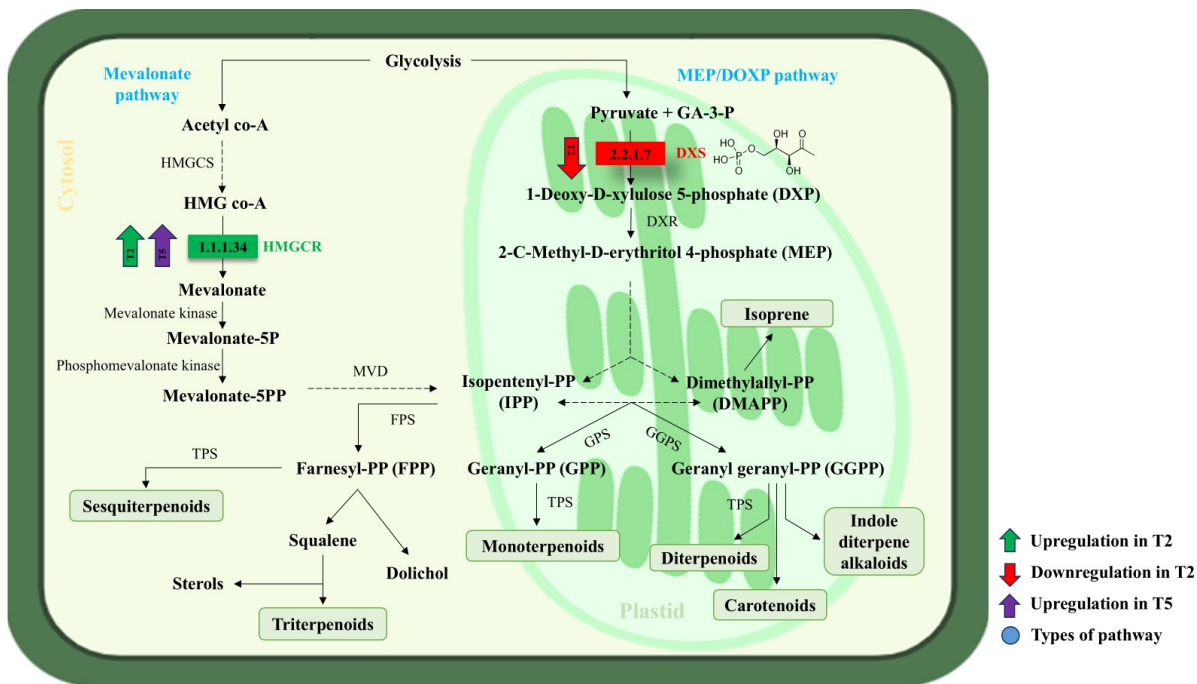


Figure 6: Overview of volatile profiling (A) (B) Venn-euler plot comparing unique and shared number of VOCs across T1, T2 and T5 sets. (C) MetaboAnalyst 6.0 generated heatmap representing common volatile organic compounds (VOCs) across experimental conditions of T1, T2 and T5. Color scale ranging from dark purple (0.6) to yellow (-0.6) represents higher to lower concentration gradients. (D) Heatmap representation of the 20 common VOCs across experimental conditions of T1 and T2 sets. Hierarchical clustering exhibits distinct VOCs expression patterns. Color scale ranging from dark purple (1) to yellow (-1) represents higher to lower concentration gradients.

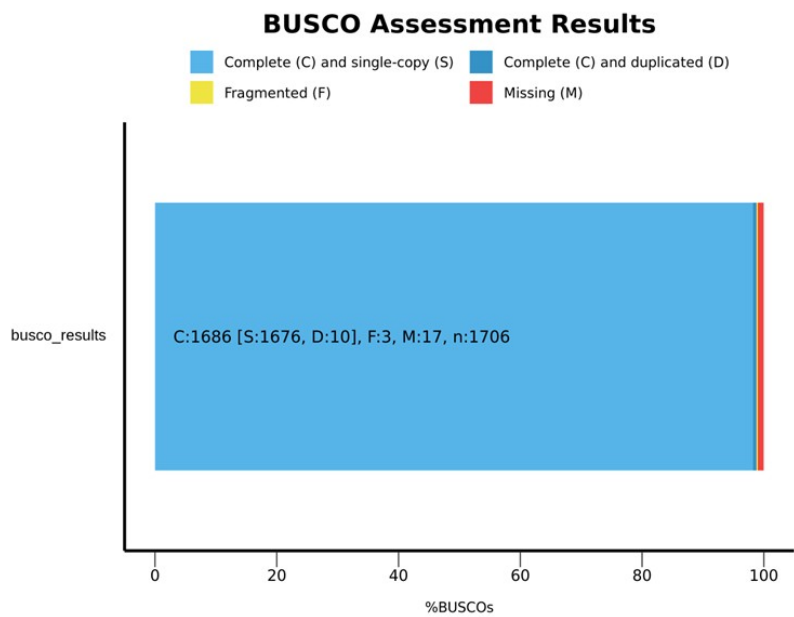
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1207 **Figure 7:** Overview of terpenoid/isoprenoid synthesis in *C. sinensis* via mevalonate (cytosolic)
1208 and non-mevalonate/MEP-DOXP (plastidial) pathway. Pathway analysis using upregulated
1209 transcripts of T1 vs T2 experimental set reveals upregulation (green upward arrow) of genes
1210 assigned to 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) and downregulation
1211 (red downward arrow) of genes assigned to 1-Deoxy-D-xylulose 5-phosphate (DXS). In the
1212 case of T1 vs T5, upregulation (purple upward arrow) of only HMGCR with no down-
1213 regulation activity of enzyme was found.



1214

Figure XX: BUSCO assessment result representing the completeness of *Trichoderma* sp. AM6 genome assembly, comprising 1706 BUSCO groups.

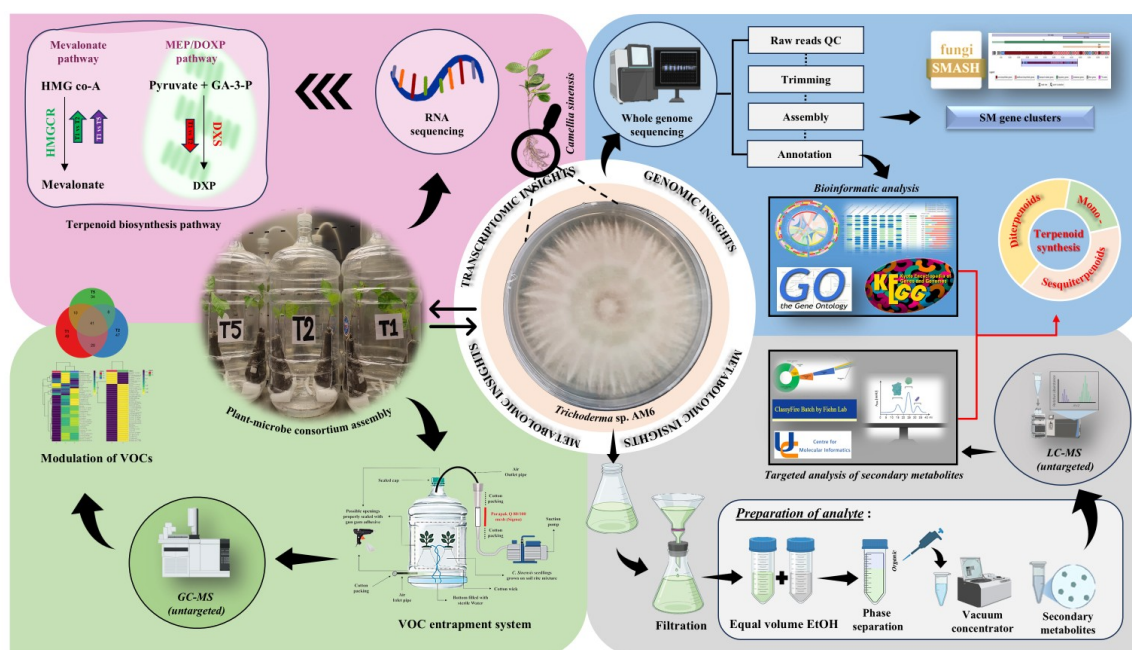


Figure: Graphical abstract