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**Research Article**

**Keywords:** Flavonoid, lignin, lipid membrane, MAPK, P absorption, water deficit

**Posted Date:** July 30th, 2026

**DOI:** https://doi.org/10.21203/rs.3.rs-10368729/v1

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**Additional Declarations:** No competing interests reported.

**Version of Record:** A version of this preprint was published at Plant Molecular Biology on October 28th, 2026. See the published version at https://doi.org/10.1007/s11103-026-01773-z.

# Transcriptomic responses of sugarcane to drought stress are modulated by bacterial synthetic communities

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# Transcriptomic responses of sugarcane to drought stress are modulated by bacterial synthetic communities

**Abstract.** Drought intensification due to climate change poses a significant constraint on global sugarcane security, as sugarcane requires substantial water for optimal growth. While Synthetic Communities (SynComs) offer potential to improve drought resilience, their application and mechanisms of action remain murky, particularly in sugarcane. Here, we used transcriptomics analysis to examine how SynComs may modulate responses to drought stress in sugarcane. We examined five potential SynComs (SC) and selected SC3, a promising consortium, for detailed gene expression analysis. This selection was based on the excellent performance of SC3-inoculated plants, as indicated by higher plant height, P uptake, and osmoprotectant content compared to other SynComs and control (C) plants. Transcriptomic analysis revealed differences in signal transduction: in SC3-inoculated plants, MAPK signaling was prominent, whereas in C plants, signaling relied more on plant hormones. Moreover, differences in energy allocation and secondary metabolite production as defense responses to drought stress were observed. SC3-inoculated plants appear to allocate energy to maintaining lipid membrane integrity and lignification, while C plants focus on antioxidant activity via flavonoids. The results indicate that bacterial SynComs inoculants can modulate drought stress responses. Overall, our study suggests that SynComs offer sustainable solutions to drought challenges in agriculture.

**Keywords:** Flavonoid, lignin, lipid membrane, MAPK, P absorption, water deficit

## Introduction

Sugarcane (*Saccharum* spp.) is a major global sugar source, accounting for about 80% of the raw material supply for sugar industries in more than 110 countries (European Commission, 2025). Despite its importance, sugarcane is highly sensitive to drought stress because it requires abundant water throughout its life cycle (dos Santos et al. 2019; Gomathi et al. 2020; Wang et al. 2025). Unfortunately, approximately 60% of its cultivation area is threatened from water deficit (Gomathi et al. 2020). Studies have shown that induced drought stress at various stages hampers sugarcane growth and development, as well as cane yields (Dinh et al. 2018; dos Santos et al. 2019; Gomathi et al. 2020). At the early stage (60 days after transplanting), agronomic and biomass traits did not recover after drought stress was over, whereas sugarcane photosynthesis did (Dinh et al. 2018). Another study showed that drought stress exposure at this

stage could reduce cane yield by about 32.9% (Gomathi et al. 2020). These trends emphasize the urgent need for strategies to enhance sugarcane resilience to drought stress.

Although various studies have demonstrated genetic control of drought response by comparing the transcriptional profiles of tolerant and sensitive sugarcane genotypes (Xu et al. 2018; Nawae et al. 2020; Contiliani et al. 2022), its further application for sugarcane breeding remains unresolved. In many plants, developing drought-resilient varieties has been limited by the complexity of resilience mechanisms and the sensitivity to environmental variables (Li et al. 2021; Prado et al. 2025). Hence, effective alternatives require a shift towards fundamental research that incorporates realistic agricultural approaches and focuses on practical applications (Ali et al. 2022; Prado et al. 2025). To survive and perform optimally under environmental stress, plants establish symbiotic interactions with microorganisms, both inside and outside their tissues (Vandenkoornhuyse et al. 2015; Wang et al. 2026). A study by Liu et al. (2021) showed that tolerant and sensitive sugarcane genotypes recruit distinct microbiome communities in response to drought stress, most likely through changes in root exudates that attract beneficial microbes (Zhalnina et al. 2018; Wang et al. 2026). This study indicates the essential role of microbial communities in modulating sugarcane response to drought stress. Thus, current attention has focused on the utilization of beneficial microbes that mediate drought resilience and nutrient use efficiency, using them as bio-stimulants or bio-fertilizers (Vargas et al. 2014; Bradacova et al. 2019; Jochum et al. 2019).

However, engineering these beneficial microbes to improve stress resilience is challenging due to the vast and intricate diversity of plant-microbe interactions (de Souza et al. 2020; Mazumder et al. 2026). To overcome such constraints, microbial inoculant approach has evolved from single-strain inoculants to consortia of multiple strains, and most recently to mimic native soil microbiomes with designed synthetic communities (SynComs) (Singh et al. 2025; Xu et al. 2025). Currently, SynComs have emerged as a powerful tool for dissecting and manipulating this symbiotic interactions by providing functional diversity, environmental stability, and improved colonization capacity (de Souza et al. 2020). However, drought resilience improvement in sugarcane remains limited to PGPR application with a single strain, such as *Bacillus spp.* and *Gluconacetobacter diazotrophicus* (Vargas et al. 2014; Chandra et al. 2018; Buqori et al. 2025). Thus, implementing SynComs to improve drought resilience in sugarcane is urgently necessary.

Studies across various plants have shown that SynComs can improve plant performance under drought stress (Hao et al. 2026; Xu et al. 2026), yet the mechanisms underlying their interactions in enhancing plant resilience to drought

remain poorly explored, particularly in sugarcane. Transcriptomic profiling has revealed extensive reprogramming of gene expression in sugarcane under drought stress (Xu et al. 2018; Nawae et al. 2020; Contiliani et al. 2022; Li et al. 2022; Wang et al. 2025). However, most studies have focused solely on plant responses in the absence of beneficial microbial inoculants. A study by Vargas et al. (2014) showed that *G. diazotrophicus* inoculation shifts gene expression, including opposing responses to abscisic acid (ABA) and ethylene signaling, thereby reducing stress in inoculated sugarcane. Hypothetically, SynComs could alter transcription profiles of sugarcane in order to reduce drought stress effects. Here, our study aimed to investigate, for the first time, how SynCom inoculation enhances drought resilience in sugarcane through physiological and transcriptomic analyses. This study highlighted the role of a specific pathway in enhancing drought resilience and sugarcane growth, offering a promising avenue for improving its resilience to drought stress.

## Materials and Methods

### Bacterial synthetic community preparation

The initial bacterial synthetic community (SynComs) used in this study were developed in a previous study (Sulastri et. al., 2026) using a modified high-throughput limiting dilution (HLD) approach (Bai et. al., 2015). Fourteen selected Syncom were cultured in National Botanical Research Institute's Phosphate (NBRIP) medium containing $FePO_4 \cdot 2H_2O$ as the insoluble phosphate source, supplemented with 30% polyethylene glycol and 1 g $L^{-1}$ of $KH_2PO_4$ during the first enrichment phase. In the second and subsequent subculture, the $KH_2PO_4$ concentration was reduced to 0.75 g $L^{-1}$ (Faller et al. 2024). Each enrichment phase was incubated for 5 days, and soluble phosphate concentration were determined at the end of incubation period using the molybdenum blue method (Murphy and Riley 1962). Once stable and effective phosphate-solubilizing activity has been achieved, the SynComs were evaluated for their plant growth promoting effect on maize seedling. Five SynComs exhibiting the highest seedling vigor index were selected for this study and further characterized for plant growth promoting traits, including production of exopolysaccharide (EPS) and hydrogen cyanide (HCN), ACC-deaminase, indole-3-acetic acid (IAA), nitrogen ($N_2$) fixation and the capacity to solubilize inorganic phosphate (Pi) and potassium (K).

The five selected SynCom were subsequently characterized by Illumina amplicon sequencing, and their bacterial composition (SC1, SC2, SC3, SC4, and SC5) are presented in Fig 1. Each SynCom was cultured on Tryptic Soy Broth (TSB) for 72 hours prior to inoculation. Pre-germinated sugarcane set (Rayungan), consisting of stalk cuttings with

sprouted buds, were inoculated by immersion in the bacterial suspension for 30 minutes. Subsequently, 10 mL of remaining bacterial culture was applied directly to each planting hole at the time of planting.

**Plant materials and experimental treatments**

Sugarcane hybrid cultivar PS862 as plant material was provided by PT Rajawali Nusantara Indonesia, East Java, Indonesia. The experiment was set in a greenhouse using a Randomized Complete Block Design (RCBD) with three factors: SynCom inoculation (6 levels), drought stress (2 levels), and P deficiency (2 levels); each combination was replicated three times (n = 3). The treatment factors marked as: 1) C (uninoculated control plants) and SC1, SC2, SC3, SC4, and SC5 (SynCom-inoculated plants), 2) WW (well-watered) and DS (drought stress), and 3) +NPK (full NPK dosage) and -NPK (Nutrient deficiency).

Inoculated sugarcane buds were planted in pots filled with 5 kg of soil. A total of 36 pots received the full NPK fertilizer dose (7 g $pot^{-1}$), while the remining 36 pots were subjected to NPK-deficiency treatment (3.5 g $pot^{-1}$) at planting time. Prior to planting, all pots were irrigated to field capacity (FC). For the drought-stress treatment, plants were maintained at FC for the first 4 weeks after planting, after which irrigation was reduced to 50% of the FC until the end of the experiment. Irrigation was applied weekly until one week before harvest. Soil moisture was monitored periodically throughout the experiment to ensure that the target moisture levels were maintained. In total, 72 pots of sugarcane plants were cultivated for 14 weeks for subsequent physiological and transcriptomic analyses. At harvest time, agronomic variables were also recorded, including plant height, leaf number, and SPAD readings.

**Physiological analyses**

After 14 weeks of drought stress and P-deficiency treatment, shoot and root samples were collected from both C and SCs sugarcane plants. The samples were rapidly frozen in liquid nitrogen and stored at -80 C until physiological analyses. This analysis included chlorophyll (Chl), total soluble sugar (TSS), phosphorus (P), catalase (CAT), and superoxide dismutase (SOD). For Chl, TSS, and P content, the results were expressed as mg $g^{-1}$ of fresh weight (fw), while CAT and SOD activity were defined as units $mL^{-1}$.

Briefly, Chl content was analyzed following Arnon's method and formula (Arnon, 1949). A 0.1 g sample of powdered leaves was mixed with 2 mL of 80% acetone and centrifuged at 14,000 × g for 5 minutes. The absorbance

of the supernatant was then measured three times using a UV-Vis spectrophotometer (Shimadzu UV-1800 Probe, Japan) at 663 nm for Chl a, 645 nm for Chl b, and 470 nm for total Chl.

Total soluble sugars (TSS) were measured using the method described by DuBois et al. (1956). A 0.1 g shoot and root powder was homogenized with 2 mL of 80% ethanol and heated at 80 °C for 1 hour. After that, about 250 µL of the supernatant was collected, combined with 250 µL of 18% phenol, and incubated for 1 hour at room temperature. The solution was then mixed with 1,450 µL of $H_2SO_4$. The TSS content was measured at 490 nm (Shimadzu UV-1800 Probe, Japan) using a glucose standard curve for reference.

For phosphorus (P) measurement, we used the molybdenum blue test as described by Murphy and Riley (1962). About 0.1 g of shoot and root powder was diluted with 10% $HClO_4$ and incubated for 30 minutes in an icebox. This solution was then centrifuged at 13,000 × rpm for 5 minutes to extract the supernatant. For the P measurement, about 300 uL of supernatant was mixed with 3 mL of ammonium molybdate and ascorbic acid solution. The mixture was incubated in the dark for 30 min. Afterward, the absorbance was measured at 882 nm using a Shimadzu UV-1800 probe (Japan). The amount of P was calculated using a standard curve generated from $KH_2PO_4$.

Crude enzyme was extracted from 0.1 g of shoot and root powder diluted in 3 mL of 50 mM Tris–HCl buffer (pH 7.0). The mixture was then centrifuged at 10,000 × g for 20 minutes at 4 °C to collect the crude extract. Afterward, CAT activity was determined using Aebi's method (Aebi, 1984). For this analysis, about 100 uL crude extract was mixed with 2.9 mL of 0.5 M phosphate buffer (pH 7.0) and 100 uL of 0.2 M $H_2O_2$ in 0.5 M phosphate buffer. The estimation of CAT activity was based on the decrease in absorbance at 240 nm due to $H_2O_2$ over 2.5 minutes. In addition, SOD activity was estimated following Kono's method (Kono, 1978). The mixture for SOD activity contained 1.3 mL of sodium carbonate buffer, 500 uL of NBT, 100 uL of Triton-X, 100 uL of hydroxylamine hydrochloride, and 70 uL of crude extract. SOD activity was measured by monitoring the color change due to NBT reduction at 560 nm over 2.5 minutes (Shimadzu UV-1800 Probe, Japan).

**Transcriptome sequencing and data analysis**

Transcriptome analysis focused on C and SC3 grown under drought stress (DS) and full NPK dosage (+NPK), with two biological replicates per treatment. At harvest time, leaf tissues from C and SC3 plants were immediately frozen in liquid nitrogen to minimize variability among individual plants. Briefly, library preparation was performed using the Collibri™ Stranded RNA Library Prep (Thermo Fisher Scientific, USA). Sequencing was performed on the

Illumina NextSeq 2000 - PE150 platform by a third-party 1st base company in Singapore. The raw RNASeq data reported in this study have been deposited under project number PRJNA1478345 in the National Center for Biotechnology Information (NCBI) database, National Library of Medicine, USA.

Raw sequencing data were converted to FASTQ format and stored. To ensure high-quality reads, the FASTQ files were processed using FASTP version 1.0.1 with default settings (Chen et al. 2018). Reads containing adapter sequences, more than 10% poly-N bases, or over 50% of bases with quality scores below Q20 were discarded. The percentages of Q20, Q30, and Q40, along with the GC content, were measured for both raw and cleaned data. Only high-quality clean reads were used for all subsequent analyses.

After quality filters, clean reads from each sample were aligned to genome-indexed sequences generated from the combined coding sequences (CDS) from *S. spontaneum* cv. AP85–441.v2018 and *S. officinarum* cv. LA-Purple.v2022, both accessed from the *Saccharum* Genome database (https://sugarcane.gxu.edu.cn/scdb/). The number of gene reads was then counted using the SALMON program version 1.11.4 (Patro et al. 2017). Afterward, differentially expressed genes (DEGs) between C and SC3 plants were assessed using DESeq2 (Love et al. 2014) with a $\log2FC \geq 1$ and p-adj $< 0.05$. Then, functional analyses of the obtained DEGs were performed using the EGGNOG, GO, and KEGG databases. DESeq2 and functional analyses were conducted using R version 4.5.0 and RStudio version 2025.05.0.

**Statistical analysis**

Agronomy and physiological data were analyzed using three-way ANOVA, followed by post hoc tests with HSD at the 5% significance level. These statistical analyses were performed using the agricolae package (Felipe de Mendiburu, 2006). Data visualization was generated using the ggplot2 and corrplot packages (Wickham, 2016). Data analyses and visualizations were performed using R version 4.5.0 and RStudio version 2025.05.0.

**Results**

**Synthetic bacterial community composition**

Characterization of plant growth-promoting (PGP) traits revealed variation among the five SynComs (Fig. 1). Overall, SC1 and SC3 showed higher PGP capabilities, followed by SC2 at a moderate level in most PGP traits, whereas SC4 and SC5 showed lower PGP activity. For nutrient metabolism, SC1, SC3, and SC5 could be prioritized, as these three

SynComs ave moderate to high capacity for N2 fixation and for solubilizing P and K. However, given our goal of developing SynCom to improve drought resilience, SC1 and SC3 showed strong potential applications with high EPS and ACC-deaminase production.

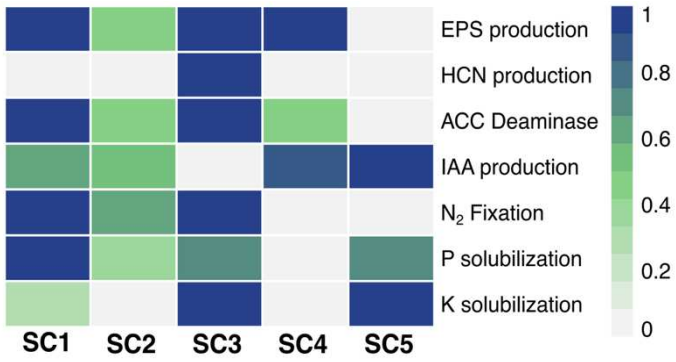



**Fig. 1.** The plant growth-promoting (PGP) traits of five SynComs (SC) were standardized and compared by z-score analysis across three replications.

Interestingly, the taxonomy analysis also showed a pattern similar to that of the PGP characterization (Fig. 2). SC3 has the most distinct genera among the others and exhibits a relatively uniform abundance pattern, with strong dominance of *Providencia*, *Brevundimonas, Phytobacter, Alcaligenes, Flavobacterium, Paenalcaligenes, and Enterobacter,* whereas *Klebsiella* and *Enterococcus* are absent. SC1 clustered more closely with SC5, indicating greater similarity in community composition, whereas SC2 was more closely related to SC3. The genera *Pseudomonas, Enterobacter,* and *Citrobacter* are highly represented in SynCom SC1, while SC2 is dominated by *Scandinavium*, *Pseudocitrobacter*, *Stenotrophomonas*, *Acinetobacter,* and *Serratia*. SynCom SC4 is enriched with *Kosakonia* and *Brevibacillus*, while SC5 is characterized by the enrichment of *Kosakonia* and *Oceanobacillus*. The number of genera uniquely enriched within each SynCom was 3 in SC1, 6 in SC2, 11 in SC3, 8 in SC4, and 2 in SC5.

**Agronomic and physiological responses to combined abiotic stresses**

Drought stress (DS) notably reduced sugarcane height of averaging 120 cm compared with 142 cm under well-watered (WW) conditions (Fig. 3A). Interestingly, SynCom inoculation significantly increased plant height, reaching 149 cm in SC3, exceeding the average height observed under WW conditions (Fig. 3B). Meanwhile, SC1 and SC5 maintained plant heights of approximately 134-137 cm, whereas SC2 and SC4 did not differ significantly from the C plants.

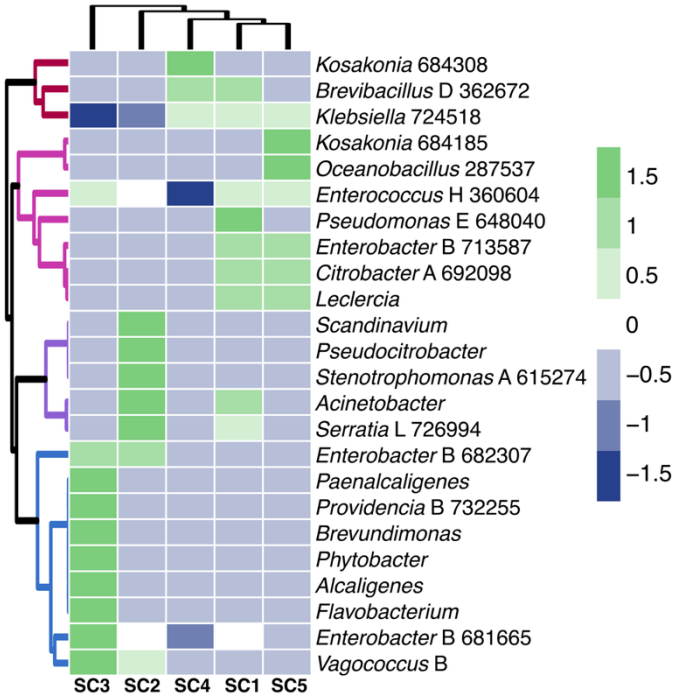



**Fig. 2.** Heatmap showing the standardized relative abundance of bacterial members in each SynCom, compared by z-score, following persistence cultivation through successive passages under P-starvation and drought stress treatment.

Phosphorus (P) and TSS analysis unveiled intriguing patterns in the effects of combined abiotic stresses (DS and -P) and SynCom inoculation on sugarcane roots and shoots. Notably, while the roots could absorb substantial amounts of P, reaching 38.08 mg $g^{-1}$, their uptake in the shoots was considerably lower at only 7.42 mg $g^{-1}$. Under -P, SC3 and SC4 demonstrated enhanced P absorption in the roots and greater uptake in the shoots compared to the other SCs (Fig. 4D-E). Additionally, distinct variations in TSS levels were observed between roots and shoots. SynCom inoculation increased TSS levels in roots, while it decreased them in shoots. SC3 and SC4 continued to exert a significant effect on increasing TSS levels in roots under DS conditions (Fig. 4F-G).

The opposite effect of SynCom inoculation on SOD and CAT activity was observed, where SynCom inoculation increased SOD activity (Fig. 4H), while it decreased CAT activity (Fig. 4I) when it compared to C plants. In particular, SC1 appeared to induce antioxidant enzyme activity to a greater extent than the other SCs. Although on average drought stress significantly degraded Chl content, its effect was inconsistent across SCs plants. However, SC1 and SC2 showed higher Chl content than other SCs and C plants, notably under DS (Fig. 4A-C).

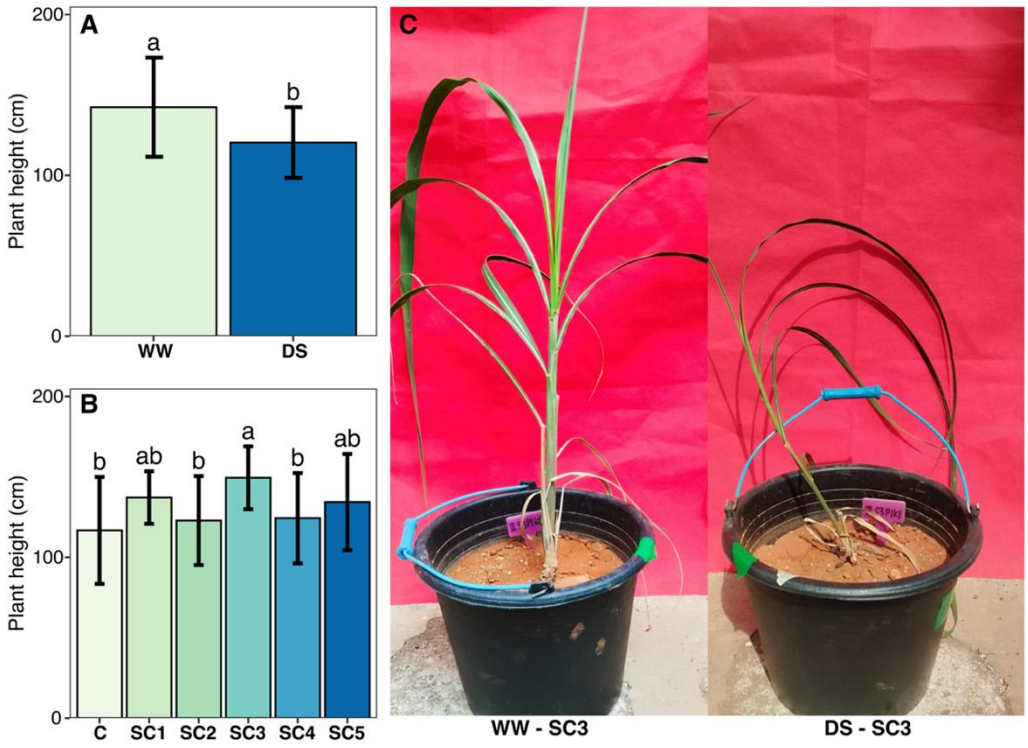



**Fig. 3.** The average of plant height at 14 weeks is influenced by (A) Drought stress, (B) SynComs inoculation, and (C) Plant phenotyping comparison between well-watered and drought-stressed plants. Significant differences ($p < 0.05$, HSD test) are indicated by different letters. Data represent mean ± SD of three replicates. WW = well-watered, DS = drought stress, C = uninoculated control plants; SC1–SC5 = SynCom-inoculated plants 1–5.

**Transcriptome analysis of control and SynCom-inoculated plants under drought stress**

Total RNA was extracted from leaf tissues of two biological replicates per treatment group: SC3 and C under drought stress and full P dosage. After robust quality filtering, clean reads were obtained with average Q20 and Q30 scores of 97.75% and 92.23%, and a GC content of 53.92% across four samples (Table S1). The proportion of mapped reads to the combined CDS of two sugarcane species ranged from 44% to 57% (Table S1). Furthermore, inter-replicate correlation analysis demonstrated strong biological reproducibility, with r values exceeding 0.89 (Fig. S1). Differentially expressed gene (DEG) analysis comparing SC3 and C plants identified 2,950 drought-responsive DEGs with log2FC ≥ 1 and p-adj < 0.05 (Fig. S1). Of these, 2,457 DEGs were down-regulated, and 495 were up-regulated in SC3 plants (Fig. S1). The global KEGG enrichment identified 28 significant functional pathways ($p \leq 0.05$), as shown in Fig. 5. Specifically, down-regulated DEGs were associated with 19 KEGG pathways, whereas up-regulated DEGs showed in only three enriched pathways.

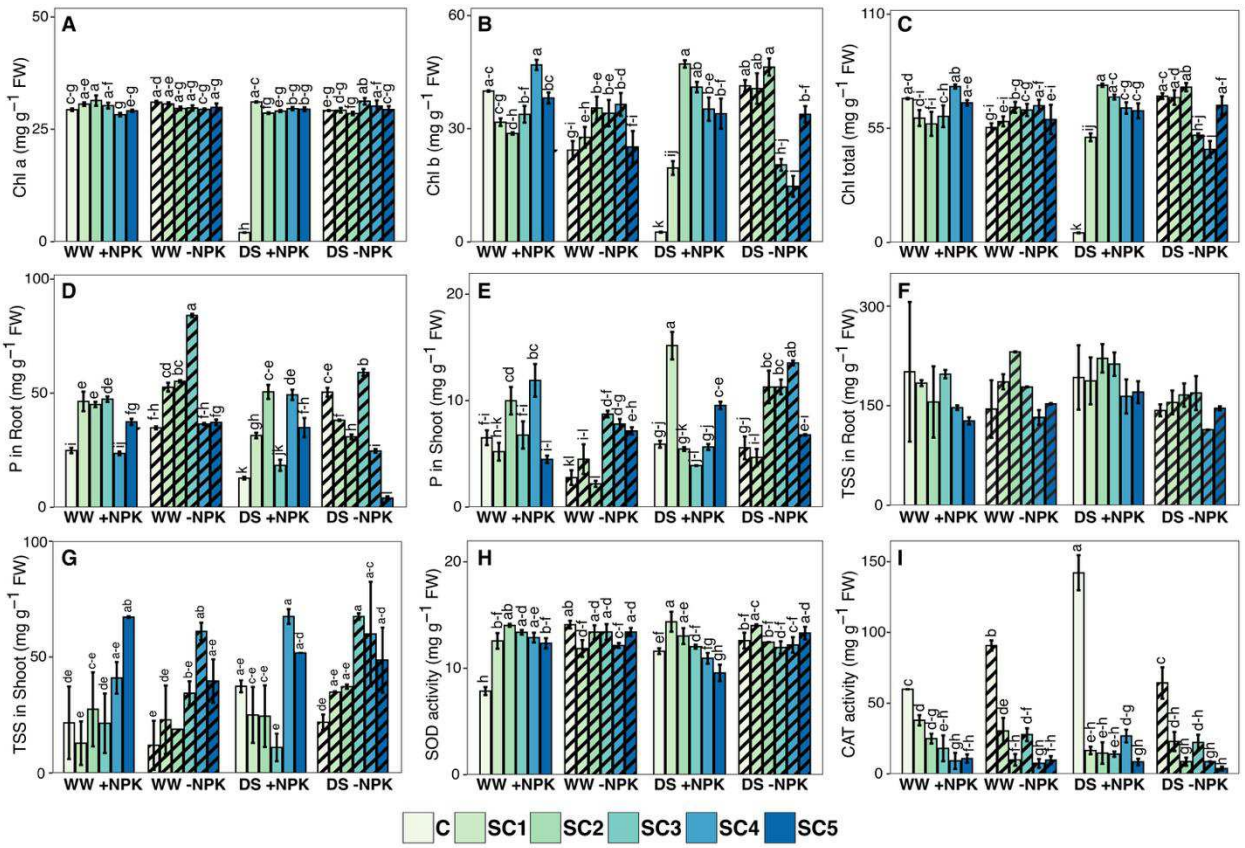



**Fig. 4**. Effects of SynComs inoculation on physiological traits of sugarcane under P deficiency and drought stress: (A) Chl a, (B) Chl b, (C) Chl total, (D) P in root, (E) P in shoot, (F) TSS P in root, (G) TSS P in shoot, (H) SOD activity, and (I) CAT activity. Significant differences ($p < 0.05$, HSD test) are indicated by different letters. Data represent mean ± SD of three replicates. WW = well-watered, DS = drought stress, +NPK = full NPK dosage, -NPK = NPK deficiency, C = uninoculated control plants; SC1–SC5 = SynCom-inoculated plants 1–5.

Notably, MAPK signaling (map04016) and plant hormone signaling (map04075) were identified in all KEGG enrichment analyses. About 27 KEGG IDs were associated with these two signal transduction pathways (Fig. 5). Five plant hormones showed signaling roles in responses to drought stress: abscisic acid (ABA), ethylene, jasmonic acid (JA), auxin, and brassinosteroid (BR) (Fig. 5). Among these, ABA, ethylene, and JA seemed to aid drought stress adaptation by mediating stomatal closure and ROS scavenging. In contrast, auxin and BR might support sugarcane growth during drought stress by promoting cell division and enlargement.

SynCom inoculation may shift growth signaling from plant hormones in C plants to MAPK in SC3 plants. This alteration is evidenced by the up-regulation of auxin- and BR-related DEGs exclusively in C plants. On the contrary, the high expression of four *glnA* genes was observed only in SC3 plants (Fig. 6). This observation was further

supported by the expression patterns at the precursor levels. MAPK signaling via *CaM* and *YUCCA* contributed to auxin biosynthesis by regulating tryptophan metabolism, which was mainly up-regulated in C plants. A similar pattern observed in BR (three) biosynthesis was exclusively up-regulated in C plants (Fig. 7).

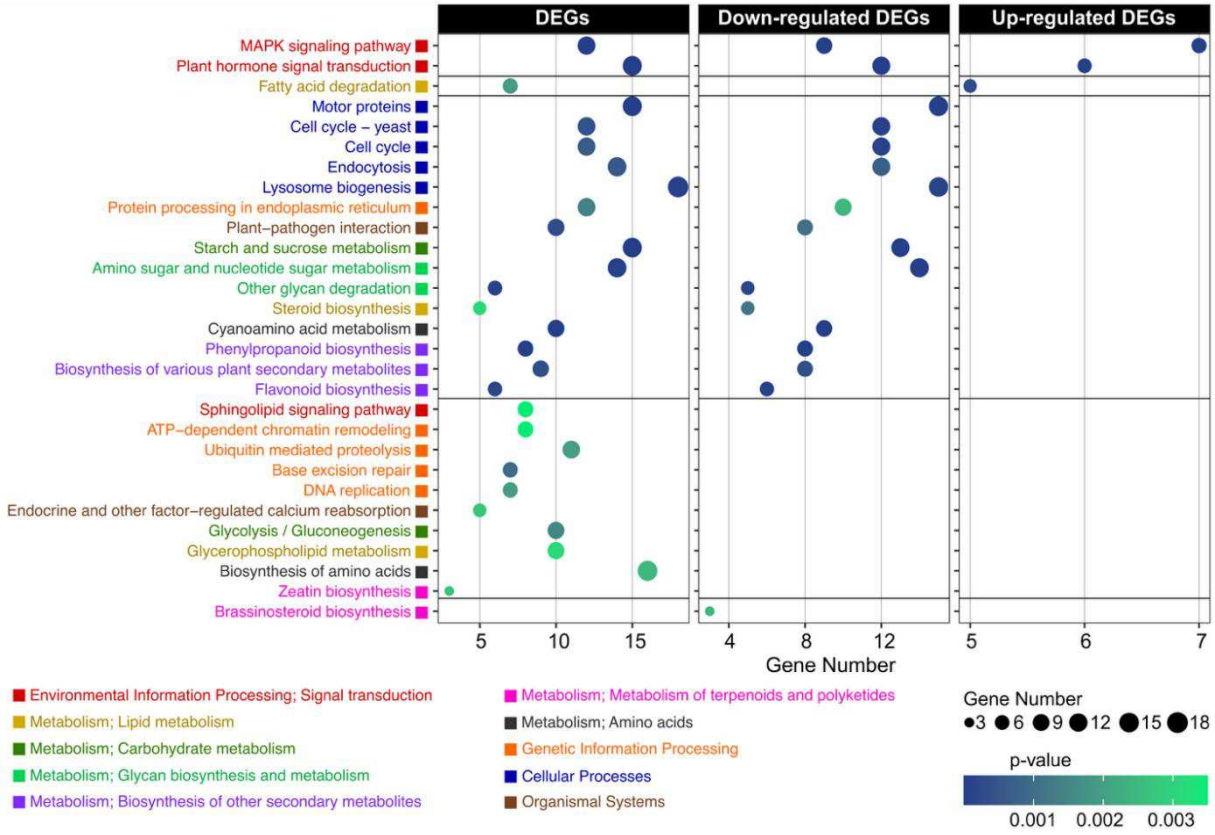



**Fig. 5.** KEGG enrichment analyses of all DEGs (2.945), down-regulated DEGs (2,457), and up-regulated DEGs (495) comparison SC3 (SynCom 3-inculated leaf) vs C (uninoculated control leaf) in sugarcane plants under drought stress.

Interconnected DEGs were found between MAPK and plant hormone signaling pathways (Fig. 6). Two key genes in MAPK signaling, *MAPKKK17*_18 and *MPK3*/6, were up-regulated in SC3 plants. The expression of *MAPKKK17*_18 genes was controlled by *SnRK2* genes from ABA signaling. Numerous studies reported that ABA accumulation is an early drought stress response, initiated by the up-regulation of *SnRK2* genes (Tajdel et al. 2016; Majeed et al. 2023). The *SnRK2* gene was up-regulated in both plants, but with distinct putative genes (Fig. 6). Additionally, *MPK3*/6 was integrated into ethylene signaling to regulate the defense response.

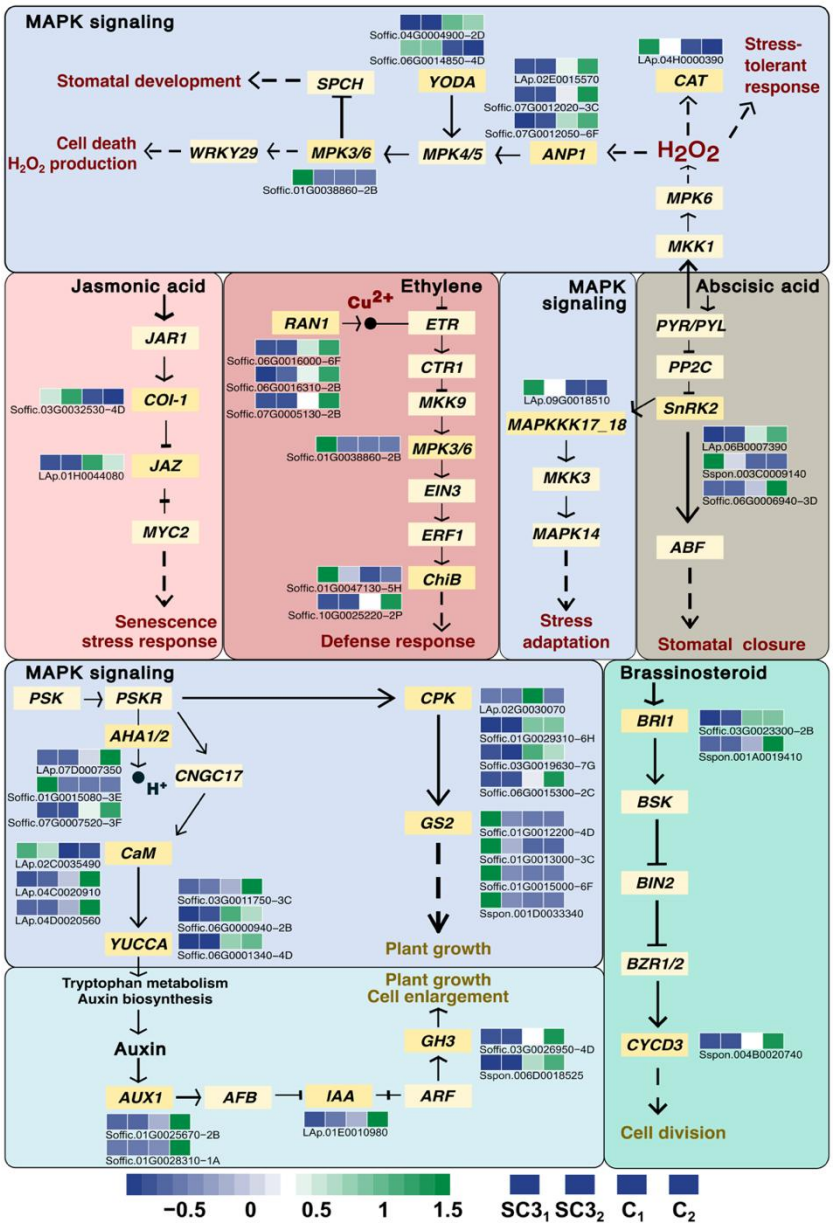



**Fig. 6.** Expression of DEGs in signal transduction pathways. The heat map displayed the log2FC values for genes in SC3 (SynCom 3-inculated leaf) vs. C (uninoculated control leaf) in sugarcane plants under drought stress.

As reported by Wang *et al.* (2025), lipid metabolism was crucial for drought resilience in sugarcane plants. Our study also highlighted the essential role of fatty acid (FA) and its derivatives metabolism (Fig. 5). Although the FA degradation pathway was enriched in up-regulated DEGs (Fig. 5), further analysis showed that C plants still have some up-regulated DEGs related to this pathway, such as *ACSL*, *CYP861A*, *MPF2*, and *ACAT* (Fig. 7). The expression profiles of some essential genes in lipid metabolism pathway, including *ADH1* and *ALDH* were up-regulated in SC3 plants, while *PLD1_2* expression was consistently down-regulated in SC3 plants (Fig. 7).

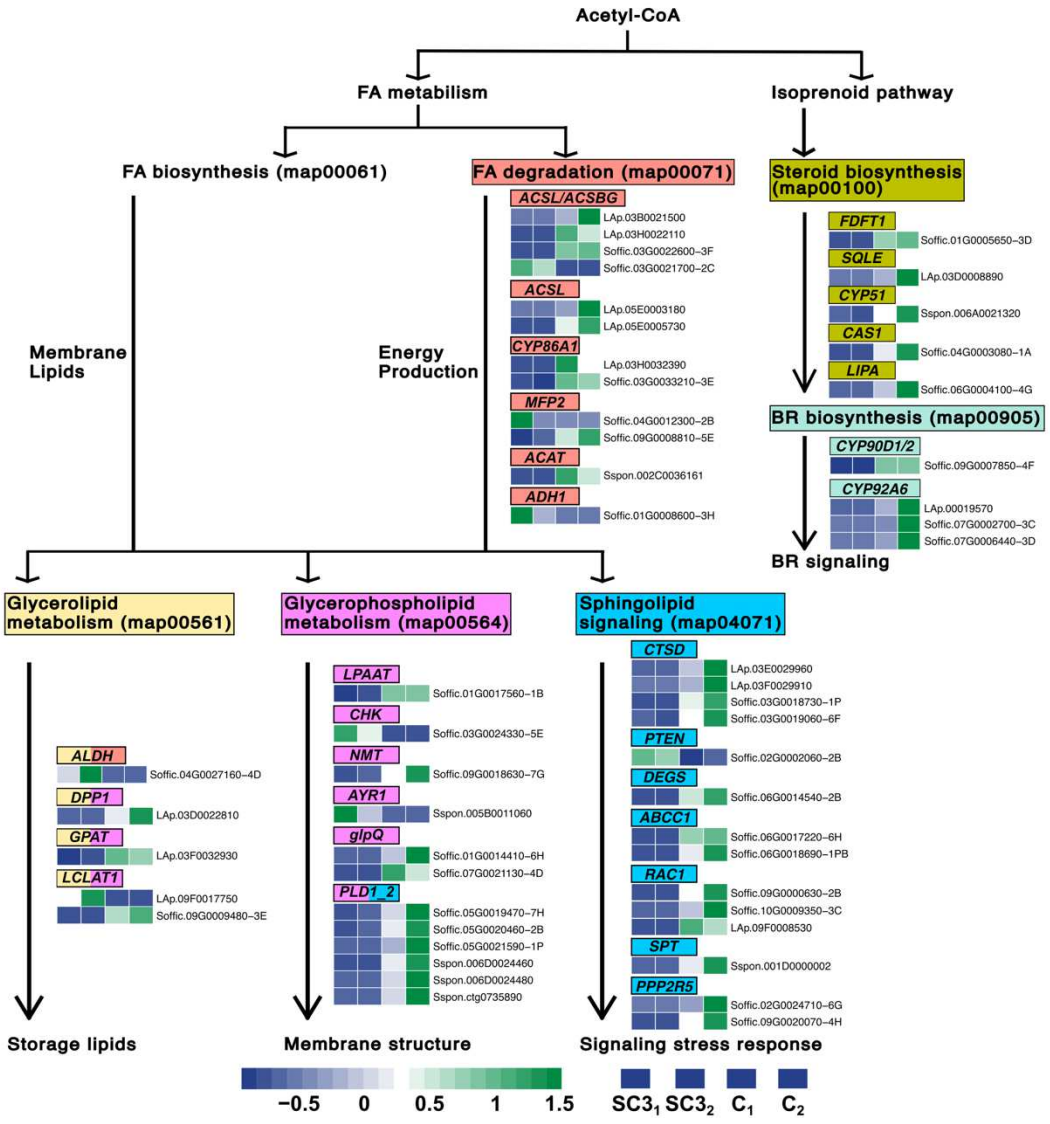



**Fig. 7.** Expression of DEGs in lipid metabolism pathways. The heat map displayed the log2FC values for genes in SC3 (SynCom 3-inculated leaf) vs. C (uninoculated control leaf) in sugarcane plants under drought stress.

Finally, we checked for changes in gene expression in pathways involved in secondary metabolite biosynthesis. All six putative *PAL* genes, as precursors to secondary metabolites, were exclusively up-regulated in C plants (Fig. 8). Interestingly, analysis of the 23 identified KEGG IDs (Fig. 5) revealed two main phenolic pathways: flavonoid metabolism (8 KEGG IDs) and lignin biosynthesis (4 KEGG IDs) (Fig. 8). Five genes related to flavonoid biosynthesis were up-regulated in C plants, such as *CHS*, *CHI*, *FH3*, *CYP75A*, and *CYP75B*. Furthermore, in the flavonoid degradation pathway, SC3 plants have up-regulated only two putative sugarcane beta-glucosidase genes, whereas C plants have up-regulated 15 genes. In lignin biosynthesis pathways, *CCR* and *CAD* were exclusively up-regulated in

C plants, whereas three putative *HCT* genes showed up-regulation in SC3 plants and only one in C plants. At the end of pathways, lignin biosynthesis is regulated by numerous putative peroxidase genes, with only 5 genes up-regulated in SC3 plants.

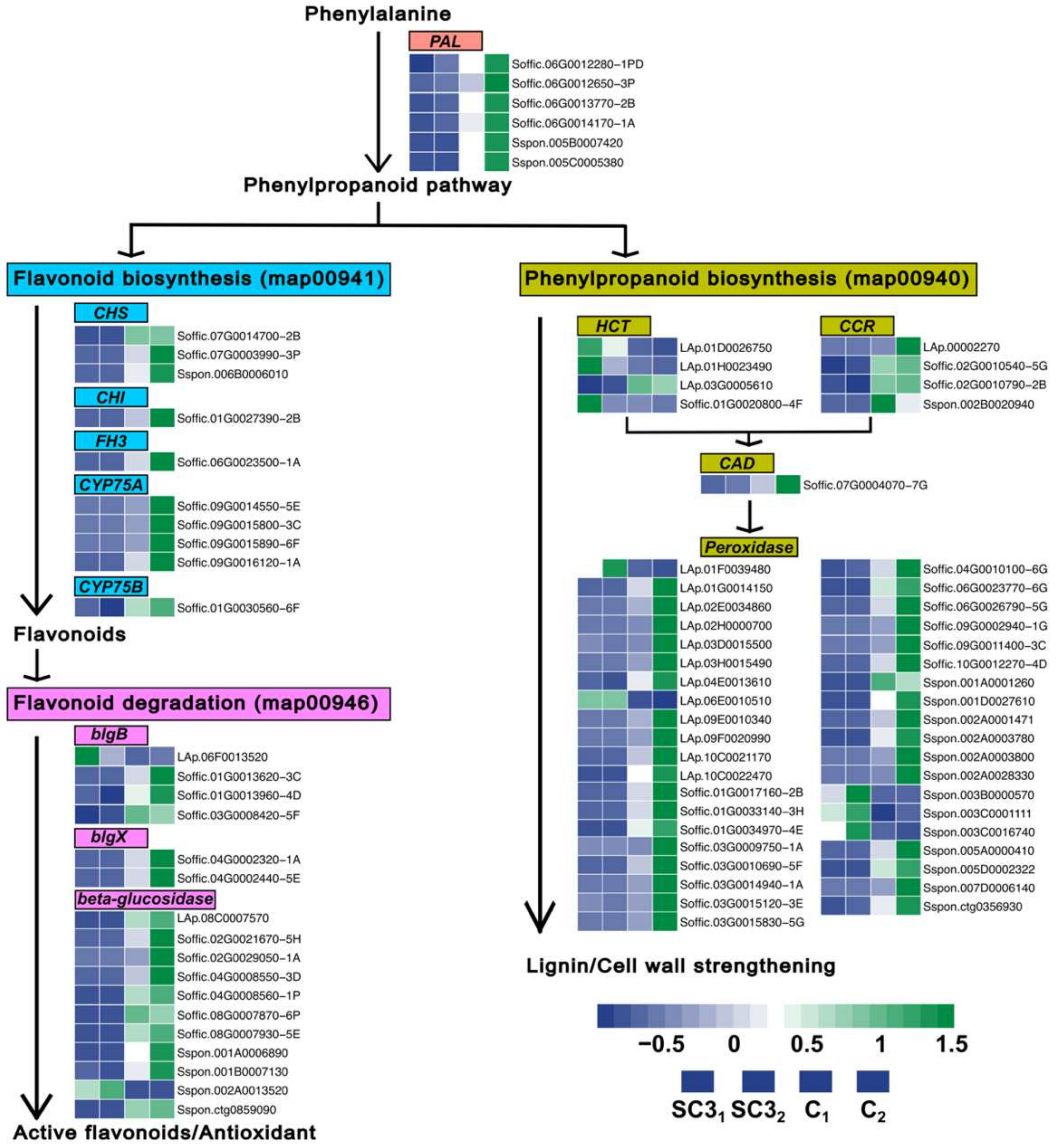



**Fig. 8.** Expression of DEGs in secondary metabolites biosynthesis. The heat map displayed the log2FC values for genes in SC3 (SynCom 3-inculated leaf) vs. C (uninoculated control leaf) in sugarcane plants under drought stress.

## Discussion

### Bacterial SynCom composition

The selection of bacterial SynCom under P-starvation and drought stress treatment after serial passages revealed distinct bacterial persistence patterns among stable synthetic communities. SC3 represent the most balanced or least differentiated SynCom, as no particular bacterial member strongly dominating the community (Fig. 1 and 2). In contrast, the enrichment of specific taxa in other SynCom may reflect superior adaptation, competitive fitness or cooperative interaction among these strains within the consortium. P-starvation and drought stress treatment acted as selective pressure that reshaped community composition over successive passages, resulting in distinct stable bacterial assemblages across SynCom. The distinct consortium composition observes across SC1-SC5 highlight how different combinations or severities of drought and P-starvation can deferentially impact on structuring stable bacterial consortia, with each SynCom demonstrating a unique persistence pattern and taxonomic structure (Rigerte et al. 2025; Zhu et al. 2025).

**SynCom inoculation enhances sugarcane growth and physiological defense under combined abiotic stresses**

Drought stress significantly reduced plant height compared with well-watered conditions, whereas NPK deficiency did not. SynCom inoculation, especially SC3, substantially increased sugarcane height, exceeding the average under well-watered conditions (Fig. 3). A modeling study by Yu et al. (2020) reported that plant height is an effective trait for predicting sugarcane yield. Any reduction in plant height would result in a decline in yield because of its correlation with cane length, a crucial trait of final sink size in sugarcane (Abu-Ellail et al. 2020; Gomathi et al. 2020). This result emphasizes the importance of SynCom in maintaining sugarcane growth and yield under drought stress.

Significant improvements in plant height may be supported by higher Chl content and P absorption in SynCom-inoculated plants compared with uninoculated controls (Fig. 4). As chlorophyll is the primary photosynthetic pigment, its reduction leads to a decline in photosynthetic capacity, thereby reducing crop yield (Muhammad et al. 2021). Chlorophyll degradation leads to leaf chlorosis, a typical drought stress symptom, consequently suppressing biomass in sugarcane (Wang et al. 2025). Sugarcane is a crop in which improved photosynthetic capacity is likely to increase yield potential (Ghannoum, 2016).

Our previous in vitro test showed that the SynCom formulas used in this study have high P-solubilization ability (Sulastri et al. 2026). This ability enables bacteria to secrete organic acids and phosphatases, which can lower soil pH and convert P into forms readily available for absorption, even under low-P conditions (Alori et al. 2017). Thus, we

observed greater P absorption in SC plants than in C plants, particularly in roots (Fig. 3), as roots directly interact with soil microbes.

**SynCom inoculation alters gene expressions of sugarcane plants under drought stress**

To investigate the effect of SynCom inoculation on drought stress resilience, we compared transcriptome profiles of uninoculated control plants (C) and SynCom-inoculated plants (SC), especially SC3, which shows higher agronomic and physiological resilience to drought stress (Fig 2 and 3). The filtering results for transcriptomic data in Table S1 and Fig. S1 confirmed the high quality of the clean reads for downstream analyses (Zhou et al. 2024; Wang et al. 2025). Global DEGs and KEGG enrichment revealed substantial differences in gene expression and its functional roles between C and SC plants.

In this study, transcriptomic analysis revealed that lipid metabolism under drought stress was significantly enriched, particularly in SC plants (Fig. 5 and Fig. 7). This result aligns with previous studies in sugarcane that reported lipid metabolism as an important resilience mechanism against drought stress (Li et al. 2022; Wang et al. 2025). Moreover, we found that aldehyde dehydrogenase (*ALDH*) and alcohol dehydrogenase (*ADH1*) genes were exclusively up-regulated in SC plants. It was previously reported that both genes were strongly up-regulated in response to drought stress across many crops, including cotton, sugarcane, tartary buckwheat, and tea. The *ALDH* and *ADH* genes mitigate elevated ROS levels under drought, thereby increasing drought resilience. For example, overexpression of ADH genes in *Arabidopsis* and tartary buckwheat, as well as *ALDH* in Arabidopsis and *Lespedeza davurica,* mitigates ROS accumulation (Zhao et al. 2017; He et al. 2025; Li et al. 2026). We also identified six putative *PLD1_2* genes that were exclusively down-regulated in SC plants. Phospholipase D (*PLD*) controls the degradation of membrane phospholipids to produce phosphatidic acid and diacylglycerol, which are up-regulated in responses to various abiotic stresses (Sun et al. 2025; Zhang et al. 2026). Thus, its down-regulation in SC plants indicates that membrane lipid degradation in sugarcane leaves during drought stress is lower after SynCom inoculation.

In this study, we identified two enriched secondary metabolites: flavonoids and lignin (Fig. 7). Flavonoids and lignin, as crucial compounds in the phenylpropanoid pathway, dynamically balance metabolic activity to enhance plant resilience to various stresses (Chen et al. 2021; Park et al. 2023; Yang et al. 2025). An evolutionary study by Zhang et al. (2021) reported that both flavonoids and lignin used p-coumaryl-CoA as a precursor. Notably, subsequent studies identified *HCT* genes as potential regulators of carbon flow at the branch point between the flavonoid and

lignin biosynthesis pathways (Chen et al. 2021; Park et al. 2023). Functional genes in various plants consistently show that overexpression of *HCT* genes leads to enhanced lignin levels and drought resilience, whereas silencing this gene results in reduced drought tolerance and plant growth (Chen et al. 2021; Ma et al. 2025). Moreover, Chen et al. (2021) reported a carbon trade-off between flavonoid and lignin biosynthesis, with increased lignin accumulation after *HCT* overexpression, while flavonoid levels decreased. These studies lead us to speculate that SynCom increases drought resilience in sugarcane by up-regulating lignin biosynthesis, as evidenced by increased HCT expression, with three of four putative *HCT* genes up-regulated in SC plants (Fig. 7). At the same time, five flavonoid biosynthesis genes were exclusively up-regulated in C plants (Fig. 7).

Together, our findings suggest that SynCom inoculation altered drought resilience mechanisms of sugarcane compared to uninoculated control plants. Transcriptomic analysis revealed that maintaining membrane lipid integrity and increasing lignin biosynthesis are the primary mechanisms underlying drought resilience in SC plants. Cell wall thickening was also observed in root tissues of wild-type sugarcane, which shows drought resilience relative to commercial sugarcane cultivars, along with up-regulation of DEGs involved in lignin, suberin, and cutin biosynthesis (Valarmathi et al. 2023). This scenario suggests drought resilience that may significantly enhance water-uptake efficiency. Moreover, ROS scavenging mechanisms in SC and C plants were regulated by distinct pathways: flavonoid biosynthesis in C plants and lipid metabolism through *ALDH* and *ADH1* genes in SC plants.

**Conclusion**

Our study suggested that SynComs better induced early growth and improved drought resilience in sugarcane plants, as reflected by higher plant height, chlorophyll content, soluble sugar content, and P absorption. Transcriptome analysis further revealed that a promising SynCom formula, SC3, showed distinct gene expression profiles under drought stress compared with uninoculated control plants. Differentially expressed genes were enriched in pathways related to signal transduction, lipid metabolism, and lignin biosynthesis, suggesting that SC3 inoculation may influence multiple biological processes involved in drought responses. This SynCom may activate drought resilience mechanisms by regulating MAPK signaling pathways, whereas uninoculated plants modulate signal transduction through plant hormone signaling pathways. Moreover, expression patterns in several key genes implied that SynCom enhanced drought resilience, likely by maintaining lipid membrane and cell wall integrity, as indicated by the up-regulation of *ALDH, ADH1*, and *HCT* in SC3 plants. In contrast, control plants seem to have regulated drought

responses through flavonoid biosynthesis, as evidenced by the upregulation of *CHS*, *CHI*, *FH3*, *CYP75A*, and *CYP75B*. This study advances our understanding of SynCom-mediated mechanisms of sugarcane drought resilience. To validate this preliminary study, investigations of SynCom's ability to colonize and interact with native soil microbiomes under diverse soil conditions were needed. Furthermore, developing advanced production methods will be crucial for the practical application of these potential SynComs amid intensified drought stress.

## Statements and Declarations


### Acknowledgment

This research was funded by the Research and Innovation for Advanced Indonesia (RIIM) competition program, jointly supported by the National Research and Innovation Agency (BRIN) and the Indonesia Endowment Fund for Education (LPDP) (Grant ID: *Gelombang 3_Sulastri*), Republic of Indonesia. The authors thank Manajemen Talenta of the National Research and Innovation Agency (BRIN) for the postdoctoral grant to Rika Sri Rahmawati.


### Conflict of interest

All authors declare there are no conflicts of interest regarding the publication of this paper.

### Author Contributions

The study conception, experimental design, and material preparation were performed by Sulastri. Primary data collection, analysis and visualization were conducted by Rika Sri Rahmawati. The first draft of the manuscript was written by Rika Sri Rahmawati and Sulastri. The manuscript was reviewed and edited by Sulastri and Rika Sri Rahmawati. Lina Herlina, Yuda Purwana Roswanjaya, and Nur Alfi Saryanah contributed to Greenhouse experiment and data collection. Nia Asiani, Ana Feronika Cindra Irawati, R. Bambang Sukmadi and Prapti Sedijani contribute to design and cultivation of SynCom. All authors read and approved of the final manuscript.

### Data availability

All authors confirm that the data supporting this study is included in the manuscript and within its Supplementary Data published online.

## References


Abu-Ellail F, Gadallah A, El-Gamal I (2020) Genetic variance and performance of five sugarcane varieties for physiological, yield and quality traits influenced by various harvest age. Journal of Plant Production 11, 519–528. doi: 10.21608/jpp.2020.102763

Aebi H (1984) Catalase in vitro. Methods in Enzymology 105, 121–126. doi: 10.1016/S0076-6879(84)05016-3

Ali S, Tyagi A, Park S, Mir RA, Mushtaq M, Bhat B, Mahmoudi H, Bae H (2022) Deciphering the plant microbiome to improve drought tolerance: Mechanisms and perspectives. Environmental and Experimental Botany 201, 104933. doi: 10.1016/j.envexpbot.2022.104933

Alori ET, Glick BR, Babalola OO (2017) Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Frontiers in Microbiology 8, 971. doi: 10.3389/fmicb.2017.00971

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiology 24, 1–15. doi: 10.1104/pp.24.1.1

Bai Y, Müller DB, Srinivas G, et al (2015) Functional overlap of the *Arabidopsis* leaf and root microbiota. Nature 528, 364–369, doi:10.1038/nature16192.

Bradacova K, Florea AS, Bar-Tal A, et al (2019) Microbial consortia versus single-strain inoculants: An advantage in PGPM-assisted tomato production? Agronomy 9, 105. doi: 10.3390/agronomy9020105

Buqori DMAI, Sugiharto B, Suherman, Siswoyo TA, Hariyono K (2025) Mitigating drought stress by application of drought-tolerant *Bacillus* spp. enhanced root architecture, growth, antioxidant and photosynthetic genes expression in sugarcane. Scientific Reports 15, 5259. doi: 10.1038/s41598-025-89457-4

Chandra P, Tripathi P, Chandra A (2018) Isolation and molecular characterization of plant growth-promoting *Bacillus* spp. and their impact on sugarcane (*Saccharum* spp. hybrids) growth and tolerance towards drought stress. Acta Physiologiae Plantarum 40, 199. doi: 10.1007/s11738-018-2770-0

Chen CT, Lu CT, Tzen JTC, Yang CY (2021) Physiological properties and molecular regulation in different edamame cultivars under drought stress. Agronomy 11, 939. doi: 10.3390/agronomy11050939

Chen S, Zhou Y, Chen Y, Gu J (2018) Fastp: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34, i884–i890. doi: 10.1093/bioinformatics/bty560

Contiliani DF, de Oliveira Nebó JFC, Ribeiro RV, Andrade LM, Peixoto Júnior RF, et al (2022) Leaf transcriptome profiling of contrasting sugarcane genotypes for drought tolerance under field conditions. Scientific Reports 12, 9153. doi: 10.1038/s41598-022-13158-5

Ding M, Zeng H, Takahashi Y, Kinoshita T, Ding H (2026) MAP kinases and stomatal regulation: Current updates and future perspectives. Trends in Plant Science 31, 420–441. doi: 10.1016/j.tplants.2025.10.013

Dinh HT, Watanabe K, Takaragawa H, Kawamitsu Y (2018) Effects of drought stress at early growth stage on response of sugarcane to different nitrogen application. Sugar Tech 20, 420–430. doi: 10.1007/s12355-017-0566-y

dos Santos CM, Endres L, da Silva ACS, et al (2019) Water relations and osmolite accumulation related to sugarcane yield under drought stress in a tropical climate. International Journal of Plant Production 13, 227–239. doi: 10.1007/s42106-019-00050-y

DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28, 350–356. doi: 10.1021/ac60111a017

de Mendiburu F (2006) Agricolae: Statistical procedures for agricultural research. R package version 1.3-7. doi: 10.32614/CRAN.package.agricolae

Faller L, Leite MFA, Kuramae EE (2024) Enhancing phosphate-solubilising microbial communities through artificial selection, Nature Communications 15, 1649, doi: 10.1038/s41467-024-46060-x

Ghannoum O (2016) How can we breed for more water use-efficient sugarcane? Journal of Experimental Botany 67, 557–559. doi: 10.1093/jxb/erw009

Gomathi R, Krishnapriya V, Arunkumar R, Govindaraj P, Ram B (2020) Physiological traits imparting drought stress tolerance to promising sugarcane (*Saccharum* spp.) clones. Plant Physiology Reports 25, 509–515. doi: 10.1007/s40502-020-00536-2

Hao X, Wang X, Wang X, et al (2026) Synthetic community derived from the root core microbes of a desert shrub Caragana korshinskii enhances wheat drought tolerance. Microbiome 14, 76. doi: 10.1186/s40168-026-02350-8

He J, Chen Y, Hao Y, et al (2025) Combining GWAS and RNA-seq approaches identifies the *FtADH1* gene for drought resistance in Tartary buckwheat. Journal of Integrative Agriculture 24, 3739–3756. doi: 10.1016/j.jia.2024.11.009

Jamshidi GK, Baghizadeh A, Karami S, Nazari M, Afroushteh M (2023) Expression of the *W36, P5CS, P5CR, MAPK3,* and *MAPK6* genes and proline content in bread wheat genotypes under drought stress. Cereal Research Communications 51, 545–556. doi: 10.1007/s42976-022-00331-9

Jochum MD, McWilliams KL, Borrego EJ, et al (2019) Bioprospecting plant growth-promoting rhizobacteria that mitigate drought stress in grasses. Frontiers in Microbiology 10, 2106. doi: 10.3389/fmicb.2019.02106

Kono Y (1978) Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Archives of Biochemistry and Biophysics 186, 189–195. doi: 10.1016/0003-9861(78)90479-4

Li J, Liu J, Zuo G, Shi H, Yin L, Wang S (2026) Molecular mechanisms of drought tolerance in Lespedeza davurica: Identification and functional characterization of a novel *ALDH* family gene (*LdALDH2-19*) in drought stress adaptation. Plant Physiology and Biochemistry 234, 111339. doi: 10.1016/j.plaphy.2026.111339

Li P, Lin P, Zhao Z, et al (2022) Gene co-expression analysis reveals transcriptome divergence between wild and cultivated sugarcane under drought stress. International Journal of Molecular Sciences 23, 569. doi: 10.3390/ijms23010569

Li P, Ma B, Palta JA, et al (2021) Wheat breeding highlights drought tolerance while ignores the advantages of drought avoidance: A meta-analysis. European Journal of Agronomy 122, 126196. doi: 10.1016/j.eja.2020.126196

Li Y, Cai H, Liu P, et al (2017) Arabidopsis *MAPKKK18* positively regulates drought stress resistance via downstream MAPKK3. Biochemical and Biophysical Research Communications 484, 292–297. doi: 10.1016/j.bbrc.2017.01.104

Liu Q, Xie S, Zhao X, et al (2021) Drought sensitivity of sugarcane cultivars shapes rhizosphere bacterial community patterns in response to water stress. Frontiers in Microbiology 12, 732989. doi: 10.3389/fmicb.2021.732989

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15, 550. doi: 10.1186/s13059-014-0550-8

Ma X, Umar AW, Zhou Y, et al (2025) Hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase *CtHCT061* enhances lignin biosynthesis and drought tolerance in safflower (*Carthamus tinctorius* L.). Industrial Crops and Products 236, 121929. doi: 10.1016/j.indcrop.2025.121929

Majeed Y, Zhu X, Zhang N, et al (2023) Harnessing the role of mitogen-activated protein kinases against abiotic stresses in plants. Frontiers in Plant Science 14, 932923. doi: 10.3389/fpls.2023.932923

Muhammad I, Shalmani A, Ali M, et al (2021) Mechanisms regulating the dynamics of photosynthesis under abiotic stresses. Frontiers in Plant Science 11, 615942. doi: 10.3389/fpls.2020.615942

Muhammad T, Zhang J, Ma Y, et al (2019) Overexpression of a mitogen-activated protein kinase *SlMAPK3* positively regulates tomato tolerance to cadmium and drought stress. Molecules 24, 556. doi: 10.3390/molecules24030556

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27, 31–36. doi: 10.1016/S0003-2670(00)88444-5

Nawae W, Shearman JR, Tangphatsornruang S, et al (2020) Differential expression between drought-tolerant and drought-sensitive sugarcane under mild and moderate water stress as revealed by a comparative analysis of leaf transcriptome. PeerJ 8, e9608. doi: 10.7717/peerj.9608

Park YJ, Kwon DY, Koo SY, et al (2023) Identification of drought-responsive phenolic compounds and their biosynthetic regulation under drought stress in *Ligularia fischeri*. Frontiers in Plant Science 14, 1140509. doi: 10.3389/fpls.2023.1140509

Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C (2017) Salmon provides fast and bias-aware quantification of transcript expression. Nature Methods 14, 417–419. doi: 10.1038/nmeth.4197

Prado K, Holland BL, McSpadden Gardener B, et al (2025) Building climate-resilient crops: Genetic, environmental, and technological strategies for heat and drought stress tolerance. Journal of Experimental Botany 76, 4395–4413. doi: 10.1093/jxb/eraf111

Singh M, Jha S, Pathak D, Maisnam G (2025) Advancing biofertilizers: The evolution from single-strain formulations to synthetic microbial communities (SynCom) for sustainable agriculture. Discover Plants 2, 226. doi: 10.1007/s44372-025-00318-w

European Commission (2025) Sugar—Agriculture and rural development. Available at https://agriculture.ec.europa.eu/farming/crop-productions-and-plant-based-products/sugar_en (accessed 17 Mei 2026).

Rigerte L, Heintz-Buschart A, Reitz T, Tarkka MT (2025) Assembly and application of a synthetic bacterial community for enhancing barley tolerance to drought. Frontiers in Bacteriology 4, 1572294. doi: 10.3389/fbrio.2025.1572294

Sulastri S, Rahmawati RS, Roswanjaya YP, et al (2026) Root colonization by synthetic bacterial communities improves below and above-ground traits in maize under combined abiotic stress. Ecological Frontiers. doi: 10.1016/j.ecofro.2026.01.006

Sun Y, Jing H, Li Z, et al (2025) Integrated transcriptomic, metabolomic and lipidomic analyses uncover the crucial roles of lipid metabolism pathways in oat (*Avena sativa*) responses to heat stress. BMC Genomics 26, 780. doi: 10.1186/s12864-025-11972-5

Tajdel M, Mituła F, Ludwików A (2016) Regulation of Arabidopsis *MAPKKK18* by *ABI1* and *SnRK2*, components of the ABA signaling pathway. Plant Signaling & Behavior 11, e1139277. doi: 10.1080/15592324.2016.1139277

Valarmathi R, Mahadeva Swamy HK, Appunu C, et al (2023) Comparative transcriptome profiling to unravel the key molecular signalling pathways and drought adaptive plasticity in shoot borne root system of sugarcane. Scientific Reports 13, 12853. doi: 10.1038/s41598-023-39970-1

Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A (2015) The importance of the microbiome of the plant holobiont. New Phytologist 206, 1196–1206. doi: 10.1111/nph.13312

Vargas L, Brígida ABS, Filho JPM, et al (2014) Drought tolerance conferred to sugarcane by association with *Gluconacetobacter diazotrophicus*: A transcriptomic view of hormone pathways. PLoS ONE 9, e114744. doi: 10.1371/journal.pone.0114744

Wang C, QuangKiet T, Li W, Jian B, Yang X (2025) Integration of transcriptome and metabolome reveals candidate metabolites responding to drought stress in sugarcane. Genomics 117, 111092. doi: 10.1016/j.ygeno.2025.111092

Wang Z, Huang H, Liu H, et al (2026) Plant-microbiota interactions under drought stress: Genetic and ecological mechanisms shaping drought-resistant rhizospheres. Plant Growth Regulation 106, 43. doi: 10.1007/s10725-026-01450-7

Wickham H (2016) Getting started with ggplot2. In: Wickham H. ggplot2: Elegant graphics for data analysis. Cham: Springer International Publishing, 11–31. doi: 10.1007/978-3-319-24277-4_2

Xu S, Cao Z, Ma K, et al (2026) A synthetic community from *Campeiostachys nutans* rhizosphere in the Qinghai-Tibet Plateau endows alfalfa with the ability to resist drought and cold. Journal of Agricultural and Food Chemistry 74, 12733–12749. doi: 10.1021/acs.jafc.5c14225

Xu S, Wang J, Shang H, et al (2018) Transcriptomic characterization and potential marker development of contrasting sugarcane cultivars. Scientific Reports 8, 1683. doi: 10.1038/s41598-018-19832-x

Xu X, Dinesen C, Pioppi A, Kovács ÁT, Lozano-Andrade CN (2025) Composing a microbial symphony: Synthetic communities for promoting plant growth. Trends in Microbiology 33, 738–751. doi: 10.1016/j.tim.2025.01.006

Yang J, Zhang Y, Jia J, Wang C, Fu Y (2025) Flavonoid-lignin crosstalk: Engineering metabolic flux for optimised plant growth and stress resilience. Plant, Cell & Environment 48, 8141–8160. doi: 10.1111/pce.70106

Yu D, Zha Y, Shi L, et al (2020) Improvement of sugarcane yield estimation by assimilating UAV-derived plant height observations. European Journal of Agronomy 121, 126159. doi: 10.1016/j.eja.2020.126159

Zhalnina K, Louie KB, Hao Z, et al (2018) Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. Nature Microbiology 3, 470–480. doi: 10.1038/s41564-018-0129-3

Zhang S, Yang J, Li H, Chiang VL, Fu Y (2021) Cooperative regulation of flavonoid and lignin biosynthesis in plants. Critical Reviews in Plant Sciences 40, 109–126. doi: 10.1080/07352689.2021.1898083

Zhang Y, Shen L, Huang Z, et al (2026) Chilling tolerance in kiwifruit alleviated by brassinosteroid: Roles in ROS scavenging, membrane lipid stabilization, and energy metabolism. Food Chemistry, 149106. doi: 10.1016/j.foodchem.2026.149106

552 Zhao J, Missihoun TD, Bartels D (2017) The role of *Arabidopsis* aldehyde dehydrogenase genes in response to high
553 temperature and stress combinations. Journal of Experimental Botany 68, 4295–4308. doi: 10.1093/jxb/erx194
554 Zhou Y, Bai YH, Han FX, et al (2024) Transcriptome sequencing and metabolome analysis reveal the molecular
555 mechanism of *Salvia miltiorrhiza* in response to drought stress. BMC Plant Biology 24, 446. doi: 10.1186/s12870-
556 024-05006-7
557 Zhu W, Han S, Cheng Y, et al (2025) Root zone microbial communities of *Artemisia ordosica* Krasch. at different
558 successional stages in Mu US Sandy Land: a metagenomic perspective with culturomics insights. Frontiers in
559 Microbiology 16, 1585700. doi: 10.3389/fmicb.2025.1585700

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