

Article

The Effects of Sugarcane Leaf Consumption by *Chilo sacchariphagus* (Lepidoptera, Crambidae) on Plant Defense Mechanisms: Transcriptomic and Metabolomic Analysis

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Abstract

Sugarcane (*Saccharum* spp.) is a globally vital sugar crop, yet its productivity faces severe challenges from infestation by *Chilo sacchariphagus*. To decipher the plant's molecular and metabolic defense mechanisms, this study applied an integrated transcriptomic and metabolomic analysis to three field-grown sugarcane cultivars (Zhongtang 4, 5, and 6) under natural borer stress. The transcriptomic analysis identified a total of 34,004 differentially expressed genes (DEGs), of which 18,674 were up-regulated, and 15,330 were down-regulated. The three cultivars exhibited distinct transcriptional regulatory patterns: Z4 and Z5 showed a global suppression-type response and a strong activation-type response, respectively, and Z6 presented a balanced-type response. A functional enrichment analysis revealed that the DEGs were significantly involved in metabolic processes, stress response, plant hormone signal transduction, phenylpropanoid biosynthesis, and plant-pathogen interaction pathways. Metabolomic analysis detected 963 differentially accumulated metabolites (DAMs), primarily including flavonoids, phenolic acids, amino acids and their derivatives, and lipids. These metabolites were significantly enriched in pathways such as amino acid metabolism, biosynthesis of secondary metabolites, and glutathione metabolism. Integrated multi-omics analysis further revealed strong synergistic regulatory relationships between gene expression and metabolite accumulation, particularly in defense-related secondary metabolic pathways, such as phenylpropanoid and flavonoid biosynthesis. Several key regulatory hubs were identified, including novel transcripts and D-xylulose-5-phosphate. Sugarcane employs a genetic background-dependent, multi-layered transcriptional reprogramming and metabolic restructuring to cope with borer stress. Cultivars Z4 and Z6 tend to activate and accumulate defensive compounds, while Z5 exhibits a different pattern of metabolic resource allocation. This research provides a systematic elucidation of the molecular mechanisms underlying insect resistance in sugarcane and offers important candidate genes and metabolites for breeding resistant varieties.



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Keywords: sugarcane; *Chilo sacchariphagus*; insect resistance; transcriptomics; metabolomics; field natural infestation; cultivar-specific resistance

1. Introduction

Sugarcane (*Saccharum* spp.) is the world's predominant source of sucrose, responsible for approximately 80% of global sugar production and over 92% of that in China [1–4]. As a cornerstone crop in southern China, it is cultivated on more than 1.2 million hectares annually, primarily in Guangxi, Yunnan, Guangdong, and Hainan provinces (autonomous regions), where it plays a vital role in regional economies and the livelihoods of millions of farmers [5–7]. Beyond its primary role in sugar production, sugarcane is also regarded as a premier feedstock for sustainable biofuel due to its exceptional biomass yield, high carbohydrate content (sucrose and fiber), and favorable energy balance [8–10].

However, sugarcane production, particularly in China, is severely constrained by various stalk borer species (e.g., *Diatraea* spp., *Chilo* spp., *Tetramesa schistaceana*), which are among the most destructive pests throughout the crop's growth cycle [5,11,12]. The sugarcane borer causes particularly severe economic losses in major producing regions. In Guangxi, a key sugarcane-producing area, approximately 40% of the cane crop suffers varying degrees of borer damage annually, leading to an estimated economic loss of around USD 147 million [13]. Similar severe losses are reported in other major sugarcane-growing countries like India and Thailand [6,7], highlighting the global significance of this pest and the urgent need for developing host-plant resistance. The damage symptoms vary with plant developmental stages. Feeding on seed canes during germination inhibits sprouting and emergence. Attacks on the growing point during the tillering stage cause “dead heart”, with survey data indicating seedling dead heart rates typically ranging from 9% to 20%, reaching 30–60% in severe cases [14]. During mid-to-late growth, larvae bore into stalks, creating extensive feeding tunnels that destroy vascular tissues, often leading to stalk breakage. At later stages, stalk infestation rates generally reach 40–60%, with severe cases exceeding 80% [14]. Ultimately, borer damage leads to sugarcane yield losses of 5.3–44.5% (averaging 25.9%) and reduces sugar content by 0.8–5.6% (averaging 2.2%) [11,15]. In addition to direct feeding damage, borer wounds facilitate the invasion of pathogens, such as *Colletotrichum falcatum* (causing red rot), compounding the losses [16]. Currently, chemical control remains the primary management strategy, relying on insecticides such as chlorpyrifos and carbosulfan. However, the efficacy of conventional chemical insecticides is often limited due to poor translocation within plant tissues [10,17]. Moreover, the long-term, excessive, and prophylactic application of these chemicals accelerates the development of pest resistance, disrupts agro-ecosystem balance, and poses risks of environmental contamination, threatening both ecosystem health and human safety.

Upon herbivore attack, plants deploy sophisticated inducible defenses, which can be categorized into direct and indirect strategies [18–20]. Direct defenses involve the biosynthesis of insecticidal compounds, antifeedants, or toxic metabolites that directly harm herbivores or deter their feeding. Indirect defenses, on the other hand, are mediated by the emission of herbivore-induced plant volatiles (HIPVs), which function as signals to attract natural enemies, such as parasitoids and predators, thereby providing a form of biological control [21–26]. These complex defensive responses are orchestrated by the dynamic reprogramming of gene expression and cellular metabolism [27]. Critically, these defense mechanisms are species-specific and can exhibit significant variation among different crop cultivars, forming the genetic and physiological basis for breeding insect-resistant varieties [28–31].

To systematically decipher these intricate defense mechanisms, modern omics technologies provide powerful, multi-dimensional tools. Within the framework of systems biology, transcriptomics and metabolomics offer complementary insights into biological processes. Transcriptomics employs high-throughput approaches, such as RNA-seq, to profile all transcripts, including messenger RNAs (mRNAs) and regulatory non-coding RNAs (ncRNAs). Quantifying expression levels and analyzing structural variations, it helps delineate gene regulatory networks and molecular mechanisms underlying specific physiological or environmental responses [31–35]. Metabolomics, in turn, provides a global analysis of small-molecule metabolites within cells, tissues, or organisms. Through qualitative and quantitative profiling—often using non-targeted approaches—it captures dynamic metabolic phenotypes that directly reflect the functional output of biological systems under genetic and environmental influences [36–40]. The integration of transcriptomic and metabolomic data is particularly powerful, enabling a systems-level exploration of crop responses to biotic stress. This approach bridges the gap between regulatory events (the “cause”) and biochemical outcomes (the “effect”). Beyond offering cross-validation to strengthen inferences, integrated multi-omics analysis—coupled with functional annotation, pathway enrichment, and molecular interaction studies—can reveal coherent mechanistic links between gene expression changes and metabolic flux alterations [41–44]. Such profiling thus facilitates the identification of key differentially expressed genes, critical metabolic pathways, and potential bioactive metabolites, generating robust hypotheses and candidate targets for subsequent experimental validation and crop improvement. However, a comprehensive understanding of the systemic defense mechanisms in sugarcane, particularly at the intersection of transcriptional and metabolic networks across cultivars with varying resistance, remains elusive.

Therefore, this study employed an integrated systems biology approach combining transcriptomics and metabolomics to systematically elucidate defense mechanisms of sugarcane in response to infestation by *Chilo sacchariphagus*. By integrating multi-omics data, we comprehensively deciphered the dynamic network of gene expression profiles and metabolite accumulation in sugarcane under insect herbivory stress. Furthermore, through omics-based correlation analysis, we systematically clarified the coordinated regulatory pathways from stress perception and signal transduction to the biosynthesis of defensive metabolites. This research not only provides critical molecular insights for mining insect-resistant genetic resources in sugarcane and developing rapid metabolic biomarkers for resistance, but also lays a solid target foundation for establishing a green and sustainable pest management system based on crop innate immunity.

2. Materials and Methods

2.1. Plant Materials and Experimental Design

Three elite sugarcane (*Saccharum*) cultivars—Zhongtang No. 4 (mid-late maturing), Zhongtang No. 5 (mid-early maturing), and Zhongtang No. 6 (ultra-early maturing)—were used in this study. These cultivars, independently developed by the Institute of Tropical Bioscience and Technology, Chinese Academy of Tropical Agricultural Sciences, were used in this study. These cultivars are widely cultivated due to their desirable agronomic traits, including high sugar content, high yield, and resistance to smut, making them suitable representative materials for investigating sugarcane stress responses.

The field trial was conducted at the Chengmai Experimental Station (110°5′ E, 20°32′ N) under a completely randomized factorial design, with two factors: cultivar (Zhongtang No. 4, No. 5, and No. 6) and borer infestation status (infested vs. control). Planting was initiated in early April 2025, with a uniform row spacing of 1.2 m to ensure consistent growth space for all plants, and the seeding rate was controlled at

45,000–52,500 buds per hectare. During planting, sufficient base fertilizer was applied, including 450 kg of urea, 750 kg of ordinary superphosphate, and 300 kg of potassium chloride per hectare. Subsequent management was standardized to minimize variability from other abiotic factors, such as irrigation, pesticide use, and mechanical damage; the field was regularly monitored to prevent confounding stressors. The experiment comprised two treatments: (1) plants naturally infested by *C. sacchariphagus* larvae during field growth (infested group), and (2) healthy plants without infestation (control group). Plant grouping was based on strict phenotypic criteria confirmed by dissection. Plants assigned to the infested group exhibited typical symptoms of borer damage, such as larval tunnels, frass, and/or dead hearts in young shoots, with the presence of *C. sacchariphagus* larvae further confirmed by leaf sheath dissection. In contrast, control plants were selected from field areas with no visible signs of borer damage, and the absence of hidden larvae was verified via leaf sheath dissection; this ensured that control plants were not attacked by arthropods or other pests.

For each cultivar, 30 plants per treatment were identified based on the above criteria and then divided into three biological replicates, each consisting of 10 randomly chosen plants from the identified set. All plants were grown under identical field conditions, and samples from infested and control groups were collected simultaneously. Leaf sheath tissues from the 10 plants within each replicate were pooled to form one biological sample, resulting in a total of 18 samples (three cultivars $\times$ two treatments $\times$ three replicates). After confirming the pest status of each plant, leaf sheath tissues were collected. All samples were immediately frozen in liquid nitrogen and stored at -80°C for subsequent transcriptomic and metabolomic analyses (Figure 1). The trial was conducted using a completely randomized design, not a split-plot arrangement.



Figure 1. Damage symptoms caused by sugarcane moth borer larvae and the larval morphology. (A,B) Typical damage symptoms in sugarcane stalks. (C) A mature larva of the moth borer.

2.2. Transcriptome Profiling

2.2.1. RNA Isolation, cDNA Library Preparation and Sequencing

Total RNA was isolated from leaf sheath samples using a modified cetyltrimethylammonium bromide (CTAB) method (Sangon Biotech, Shanghai, China) in combination with PBIOZOL Reagent (Bioer Technology, Hangzhou, China) [45]. RNA integrity was assessed using a Qsep400 bioanalyzer (Bioptic, Taiwan, China), and concentration was quantified using a Qubit[®] 4.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) with the Qubit[®] RNA Assay Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Only samples with an RNA Integrity Number (RIN) > 7.0 , an A260/A280 ratio between 1.8 and 2.2, and an A260/A230 ratio > 2.0 were used for downstream processing. Poly(A)⁺ mRNA was enriched from total RNA using oligo(dT) magnetic beads (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) and then fragmented. First-strand cDNA synthesis was performed using random hexamer primers and the FastQuant RT Kit (Tiangen, Beijing, China), followed by second-strand synthesis. The double-stranded cDNA was subjected to

end repair, poly(A) tailing, and adapter ligation to construct sequencing libraries. Library quality was checked using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). Paired-end sequencing (PE150) was performed on an MGI-Seq 2000 platform at Metware Biotechnology Co., Ltd. (Wuhan, China).

2.2.2. Sequencing Data Processing, Quality Control, and Genome Alignment

Raw sequencing reads were processed with fastp (v0.23.2) to remove adapters and low-quality bases with default parameters for paired-end reads [46]. The filtering criteria included: removal of adapter-containing reads; discarding paired-end reads if either read contains >10% undetermined bases (N) or >50% low-quality bases (Phred score $\leq$ 20). After filtering, clean reads were obtained, and basic statistics, including Q20, Q30, and GC content, were calculated (Table 1). The resulting high-quality clean reads were used for subsequent analyses.

Table 1. Sample transcriptome sequencing data.

Sample	Raw Reads	Raw Base (G)	Clean Reads	Clean Base (G)	Error Rate (%)	Q20 (%)	Q30 (%)	GC Content (%)	Alignment Rate (%)
Z4-CK1	59,292,670	8.89	57,686,678	8.65	0.02	98.76	96.14	52.27	93.78%
Z4-CK2	46,421,646	6.96	44,864,886	6.73	0.02	98.84	96.38	51.78	93.99%
Z4-CK3	42,163,176	6.32	40,712,060	6.11	0.02	98.73	96.06	53.17	93.97%
Z4-T1	59,171,604	8.88	56,772,098	8.52	0.02	98.82	96.29	51.69	73.21%
Z4-T2	47,688,920	7.15	44,390,364	6.66	0.02	98.77	96.20	54.19	89.59%
Z4-T3	69,472,648	10.42	67,362,928	10.1	0.02	98.66	95.82	51.95	89.36%
Z5-CK1	52,318,658	7.85	50,985,106	7.65	0.02	98.92	96.65	51.92	94.22%
Z5-CK2	54,530,248	8.18	53,174,580	7.98	0.02	98.84	96.38	51.96	94.37%
Z5-CK3	45,430,408	6.81	44,062,066	6.61	0.02	98.77	96.15	52.12	94.35%
Z5-T1	56,961,986	8.54	54,841,094	8.23	0.02	98.93	96.69	53.10	92.11%
Z5-T2	59,144,626	8.87	56,992,188	8.55	0.02	98.97	96.80	53.13	92.16%
Z5-T3	49,602,910	7.44	48,382,216	7.26	0.02	98.74	95.98	52.21	92.95%
Z6-CK1	47,542,408	7.13	46,020,560	6.9	0.02	98.79	96.25	52.32	93.97%
Z6-CK2	45,365,036	6.8	41,203,824	6.18	0.02	98.63	95.89	55.65	93.80%
Z6-CK3	46,060,858	6.91	44,613,568	6.69	0.02	98.68	95.86	51.95	94.14%
Z6-T1	79,734,882	11.96	76,227,130	11.43	0.02	98.98	96.82	52.51	92.73%
Z6-T2	42,232,708	6.33	40,956,706	6.14	0.02	98.91	96.61	52.21	92.64%
Z6-T3	44,278,114	6.64	42,913,660	6.44	0.02	98.65	95.82	52.21	92.70%

Clean reads were aligned to the sugarcane reference genome *Saccharum spontaneum* (Soffic.genome-hic20191009.fasta), downloaded from <https://sugarcane.gxu.edu.cn/scdb/genomics/genome/lap> (accessed on 12 July 2025) using HISAT2 (v2.2.1) [47]. The corresponding genome annotation file (GTF format) was utilized to guide transcript assembly during the alignment process.

2.2.3. Data Analysis

Based on the alignments generated by HISAT2, novel transcripts were assembled using StringTie (v2.1.6) [48]. Read counts mapped to each gene were calculated using featureCounts (v2.0.3), and gene expression levels were quantified and normalized as Fragments Per Kilobase of transcript per Million mapped fragments (FPKM). Differential gene expression analysis between different treatment groups was performed using DESeq2 (v1.22.1) [49]. Significantly differentially expressed genes (DEGs) were identified with a threshold of a Benjamini-Hochberg adjusted false discovery rate (FDR) < 0.05 and an absolute log₂ fold change ($|\text{Log}_2\text{FC}| \geq 1.0$). Finally, functional enrichment analysis of the identified DEGs was conducted. The Kyoto Encyclopedia of Genes and Genomes (KEGG) [50] pathways and Gene Ontology (GO) [51] terms were analyzed for enrichment using the hypergeometric test implemented in the clusterProfiler R package (v4.6.0). Terms with an adjusted *p*-value (padj) < 0.05 were considered significantly enriched.

2.3. Metabolome Profiling

2.3.1. Sample Preparation

The biological samples were initially freeze-dried using a vacuum lyophilizer (Scientz-100F, Scientz, Ningbo, China). The dried samples were then ground into a fine powder using a grinder (MM 400, Retsch GmbH, Haan, Germany) at 30 Hz for 1.5 min. Subsequently, 30 mg of the powder was accurately weighed (using an MS105DM electronic balance, Mettler-Toledo, Greifensee, Switzerland) and extracted with 1500 μ L of a pre-cooled (-20°C) 70% methanol aqueous solution containing internal standards (maintaining a fixed solvent-to-sample ratio of 1500 μ L per 30 mg). The mixture was subjected to intermittent vortexing (for 30 s every 30 min), repeated for a total of six cycles. After centrifugation at 12,000 rpm for 3 min, the supernatant was collected, filtered through a 0.22 μ m microporous membrane, and transferred to an injection vial for subsequent UPLC-MS/MS analysis [52,53].

2.3.2. UPLC Conditions

All samples were analyzed in sequence by the LC-MS system. The chromatographic separation was performed on a Waters ACQUITY UPLC system equipped with an HSS T3 column (1.8 μ m, 2.1 mm $\times$ 100 mm) maintained at 40°C . The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B), delivered at a flow rate of 0.40 mL/min. The injection volume was 4 μ L. The gradient elution program was set as follows: 0–2.1 min, 95% A to 25% A (linear); 2.1–4.1 min, 25% A to 1% A (linear); 4.1–5.6 min, hold at 1% A; and 5.6–5.7 min, 1% A to 95% A (linear), followed by re-equilibration at 95% A for 1.4 min [53,54].

2.3.3. MS Conditions

Data were acquired using a data-dependent acquisition (DDA) mode, which cycled between a full-scan MS survey and subsequent MS/MS scans of the most intense precursor ions. Dynamic exclusion was enabled to improve spectral quality across lower-abundance ions. Electrospray ionization was operated in both positive and negative polarities. Full-scan spectra (m/z 84–1250) were acquired at a resolution of 35,000. The key instrumental parameters were set as follows: ion spray voltage, 3.5 kV (positive) or 3.2 kV (negative); sheath gas flow rate, 30 arb.; auxiliary gas flow rate, 5 arb.; ion transfer tube temperature, 320°C ; vaporizer temperature, 300°C ; stepped normalized collision energy (NCE), 30, 40, and 50%; intensity threshold for triggering MS/MS, 1×10^6 cps; maximum number of dependent scans (Top N), 10; and dynamic exclusion duration, 3 s [55].

2.3.4. Metabolome Data Analysis

Multivariate statistical analysis was employed to investigate the metabolic profiles. An unsupervised principal component analysis (PCA) [56] was first conducted on unit variance-scaled data using the `prcomp` function in R to assess inherent sample clustering and overall data structure. To identify differentially abundant metabolites between comparison groups, supervised orthogonal projections to latent structures-discriminant analysis (OPLS-DA) [57] was subsequently performed. The OPLS-DA model was constructed using the `MetaboAnalystR` [58] package after $\log_2$ -transformation and mean-centering of the data, and its validity was evaluated through 200-iteration permutation testing to guard against overfitting. Metabolites with a variable importance in projection (VIP) score > 1.0 and an absolute $\log_2$ -fold change ($|\log_2\text{FC}| \geq 1.0$) were regarded as statistically significant. Finally, all identified metabolites were functionally annotated and mapped to biological pathways using the KEGG Pathway database (<http://www.kegg.jp/kegg/compound/>, accessed on 12 July 2025) via the KEGG Compound database.

3. Results

3.1. Transcriptome Analysis

3.1.1. Transcriptome Sequencing Data Quality Control

The transcriptome sequencing data for all samples are summarized in Table 1. Following quality control, approximately 42.16 to 79.73 million raw reads were generated per sample, yielding 40.71 to 76.23 million high-quality clean reads after filtering. All libraries exhibited high sequencing quality, with Q20 and Q30 scores consistently above 98.60% and 95.80%, respectively, and GC contents ranging from 51.69% to 54.19%. Alignment of clean reads to the reference genome resulted in mapping rates of 73.21% to 94.37%. Pearson correlation analysis of the gene expression profiles demonstrated high reproducibility among biological replicates within each variety and distinct genotype-specific responses to borer infestation (Figure 2). Collectively, these metrics indicate that the sequencing data from all biological replicates are of sufficient depth and high quality, with normal base composition and high alignment rates, thereby validating their suitability for subsequent bioinformatic analyses.

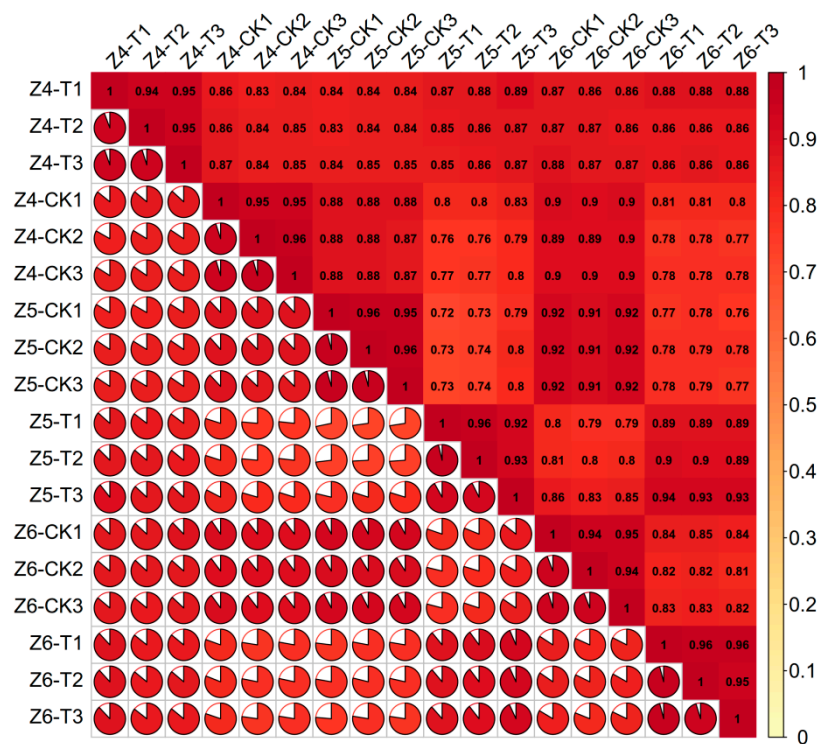


Figure 2. Pearson correlation heatmap of gene expression profiles across samples from three sugarcane varieties (Z4, Z5, and Z6) under healthy (control, CK1–CK3) and sugarcane borer-infested (treatment, T1–T3) conditions. Rows and columns represent individual samples. The color gradient and circular markers denote correlation strength (0 = yellow, weak; 1 = dark red, strong), revealing high expression reproducibility within varieties and genotype-specific responses to borer infestation.

3.1.2. Identification of Differentially Expressed Genes (DEGs)

Differentially expressed genes (DEGs) between the natural infestation group and non-infestation group samples were screened using thresholds of $|\log_2(\text{Fold Change})| \geq 1$ and a false discovery rate (FDR) < 0.05 . Global analysis revealed (Figure 3) that a total of 34,004 DEGs were detected. Among these, 18,674 genes (54.92% of total DEGs) were significantly up-regulated, while 15,330 genes (45.08%) were significantly down-regulated. The number of up-regulated genes was notably higher than that of down-regulated genes. Furthermore, 71,212 genes showed no significant change in expression. These results

indicate that natural infestation triggered extensive transcriptional reprogramming in the host, involving widespread gene activation and suppression.

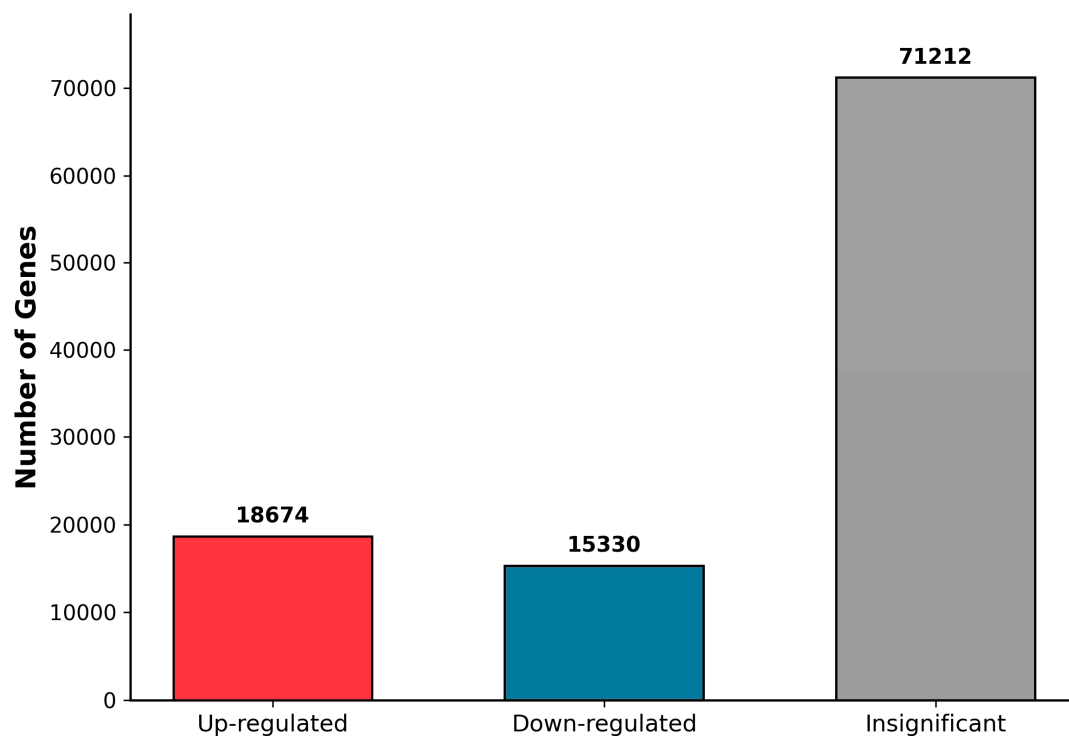


Figure 3. Statistical bar chart of the number of genes with different expression patterns. Red indicates significantly up-regulated genes, blue indicates significantly down-regulated genes, and gray indicates genes with no significant expression difference. The specific number of genes in each category is labeled on the top of the corresponding column.

Under stem borer stress, the top 10 most significantly upregulated and downregulated genes exhibited distinct expression patterns as listed in Table 2. The upregulated genes showed $\log_2FC$ values ranging from 9.72 to 11.17 (corresponding to approximately 950–2600-fold increases in expression), with adjusted p -values (p_{adj}) all below 1×10^{-12} . The gene *Soffic.07G0006920-5P* (CK: 0.23; T: 629.85; $\log_2FC = 11.17$) and its homolog *Soffic.07G0006920-4P* both displayed a typical “silenced-to-activated” expression pattern, suggesting that this gene family may play a key role in the stress response. Furthermore, 40% of the upregulated genes (4 out of 10) were novel genes, all characterized by low basal expression (CK < 1.0) and strong induced expression under stress (T > 100), indicating their potential involvement in stress-specific responses.

The top 10 most significantly downregulated genes had $\log_2FC$ values between −9.21 and −7.41 (expression reduced to 0.0064–0.018-fold of the control), with p_{adj} values all below 1×10^{-6} . Among them, *Soffic.08G0006920-3G* (CK: 318.10; T: 0.55) showed the greatest downregulation magnitude, while the novel gene *novel.43204* ($p_{adj} = 5.11 \times 10^{-57}$) exhibited the highest statistical significance. Only 10% of these downregulated genes were novel, with the remainder being known functional genes that were highly expressed under control conditions (>30). This suggests that the borer stress primarily suppresses conserved, highly expressed functional genes under normal physiological conditions.

Table 2. Top 10 most significantly upregulated and downregulated differentially expressed genes (DEGs) in sugarcane varieties under sugarcane borer infestation compared with healthy control plants.

Gene ID	Control Group Expression (CK)	Treatment Group Expression (T)	Fold Change	Log ₂ Fold Change	Adjusted <i>p</i> -Value (padj)	Type
Soffic.07G0006920-5P	0.23	629.85	2695.04×	11.17	3.79×10^{-39}	up
novel.31571	0.12	496.59	4114.17×	11.15	1.07×10^{-42}	up
Soffic.07G0006920-4P	0.00	345.65	34,564.53×	10.94	1.32×10^{-12}	up
Soffic.06G0018990-4P	0.36	497.37	1392.54×	10.51	1.43×10^{-43}	up
novel.31575	0.21	318.58	1511.68×	10.18	2.45×10^{-36}	up
novel.34925	0.00	182.17	18,216.78×	10.02	1.04×10^{-26}	up
Soffic.08G0015240-2T	0.00	181.86	18,186.21×	10.02	2.04×10^{-36}	up
Soffic.08G0015220-5P	0.09	201.24	2270.63×	9.84	6.94×10^{-36}	up
Soffic.01G0022660-1D	0.00	161.13	16,113.49×	9.84	1.18×10^{-32}	up
novel.15140	1.23	1056.33	856.32×	9.72	2.87×10^{-50}	up
Soffic.08G0004860-3G	318.10	0.55	0.0017×	−9.21	3.56×10^{-34}	down
Soffic.04G0027170-2C	123.60	0.22	0.0018×	−8.77	2.76×10^{-28}	down
Soffic.10G0008190-2D	71.64	0.11	0.0015×	−8.30	1.27×10^{-21}	down
novel.43204	617.45	1.92	0.0031×	−8.16	5.11×10^{-57}	down
Soffic.03G0009410-1B	76.21	0.22	0.0029×	−8.07	5.23×10^{-06}	down
Soffic.05G0014220-5F	244.34	1.25	0.0051×	−7.58	1.38×10^{-38}	down
Soffic.06G0004770-1B	86.47	0.50	0.0057×	−7.55	4.03×10^{-08}	down
Soffic.10G0009880-2B	33.82	0.00	0.0000×	−7.53	5.24×10^{-21}	down
Soffic.07G0026870-5E	61.28	0.25	0.0041×	−7.44	5.26×10^{-18}	down
Soffic.03G0025860-1A	185.25	1.18	0.0064×	−7.41	1.43×10^{-43}	down

Note: The table presents the top 10 upregulated and downregulated DEGs, ranked by absolute log₂fold change and statistical significance (adjusted *p*-value < 0.05). Gene ID: Unique identifier for each transcript/gene. Control group expression (CK): Normalized gene expression level in healthy plants. Treatment group expression (T): Normalized gene expression level in plants infested by sugarcane borer. Fold change: Ratio of treatment to control expression. log₂Fold Change: Log₂-transformed fold change; positive values indicate upregulation, and negative values indicate downregulation. Adjusted *p*-value (padj): Statistically significant threshold after multiple test correction. Type: Direction of differential expression (up = upregulated; down = downregulated).

3.1.3. Transcriptional Profiling of Three Sugarcane Cultivars Revealed by Gene Expression Clustering Analysis

The three sugarcane cultivars exhibited three distinct transcriptional regulatory strategies in response to borer infestation (Figure 4). The suppressive-response type (Z4) demonstrated global transcriptional repression. Infestation led to the downregulation of 68% of its differentially expressed genes (DEGs), with 23 genes being strongly suppressed (Z-score < −2), indicating a defense strategy primarily reliant on “turning off” gene expression. The activating-response type (Z5) showed the most pronounced transcriptional activation, with 17.5% of its DEGs (35 genes) being strongly upregulated (Z-score > 2)—the highest proportion among the three cultivars—suggesting an aggressive response involving the large-scale activation of gene expression. The balanced-response type (Z6) displayed a moderate, mixed regulatory pattern. The numbers of upregulated and downregulated genes were balanced, the magnitude of expression changes was generally small, and no significant clusters of strongly activated or suppressed genes were observed, reflecting a more refined and balanced transcriptional regulatory mode. The genetic background determines the core transcriptional strategy of sugarcane in responding to borer stress, manifesting as three distinct pathways: the suppressive type represented by Z4, the activating type represented by Z5, and the balanced type represented by Z6.

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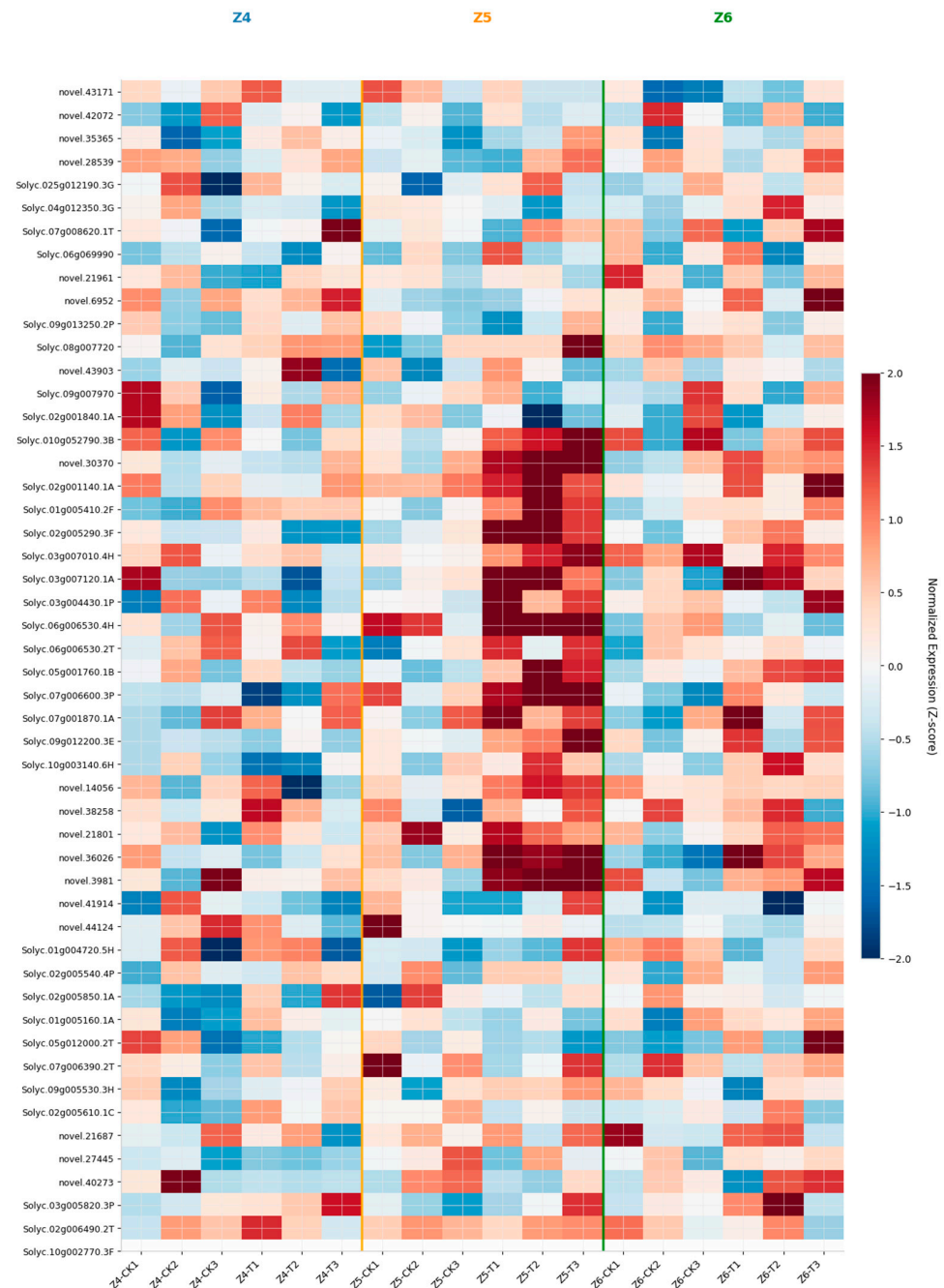


Figure 4. Clustering heatmap of differentially expressed genes (DEGs) in three sugarcane cultivars (Z4, Z5, and Z6) under treatment (T) and control (CK) conditions. Expression patterns of the top 200 DEGs, selected by coefficient of variation across 34,004 detected genes, are shown. Gene expression was normalized by Z-score (−2 to 2). Each column represents a sample, grouped by cultivar (Z4, Z5, and Z6) and treatment (CK: control; T: treated). Red and blue indicate expression above and below the mean, respectively.

3.1.4. Differentially Expressed Genes GO Analysis

To elucidate the biological significance of transcriptional changes in sugarcane plants under stem borer stress, we performed Gene Ontology (GO) enrichment analysis on the differentially expressed genes (DEGs). The results showed that a total of 34,004 DEGs were significantly enriched in 46 functional categories, spanning the three major GO domains (Figure 5): biological process, cellular component, and molecular function. In the cellular component domain, the DEGs were primarily localized to the cellular anatomical entity (23,422 genes) and protein-containing complex (2918 genes). Under the Biological Process

domain, these genes were widely involved in 20 functional categories, including metabolic process, cellular process, response to stimulus, biological regulation, developmental process, multicellular organismal process, as well as immune system process, signaling, and reproductive process. Among them, the most significantly enriched terms in terms of gene number included cellular process (17,535 genes), metabolic process (15,125 genes), response to stimulus (8954 genes), biological regulation (6804 genes), regulation of biological process (6276 genes), developmental process (3666 genes), and multicellular organismal process (3088 genes). In the Molecular Function domain, the DEGs were mainly enriched in 24 functional categories, including binding (15,811 genes), catalytic activity (14,033 genes), structural molecule activity, transporter activity, transcription regulator activity, and molecular transducer activity. Among these, binding and catalytic activity were the most predominant categories in terms of gene number, indicating that the molecular functional response of sugarcane to stem borer stress is highly concentrated in fundamental biological processes, such as protein interaction and metabolic regulation.

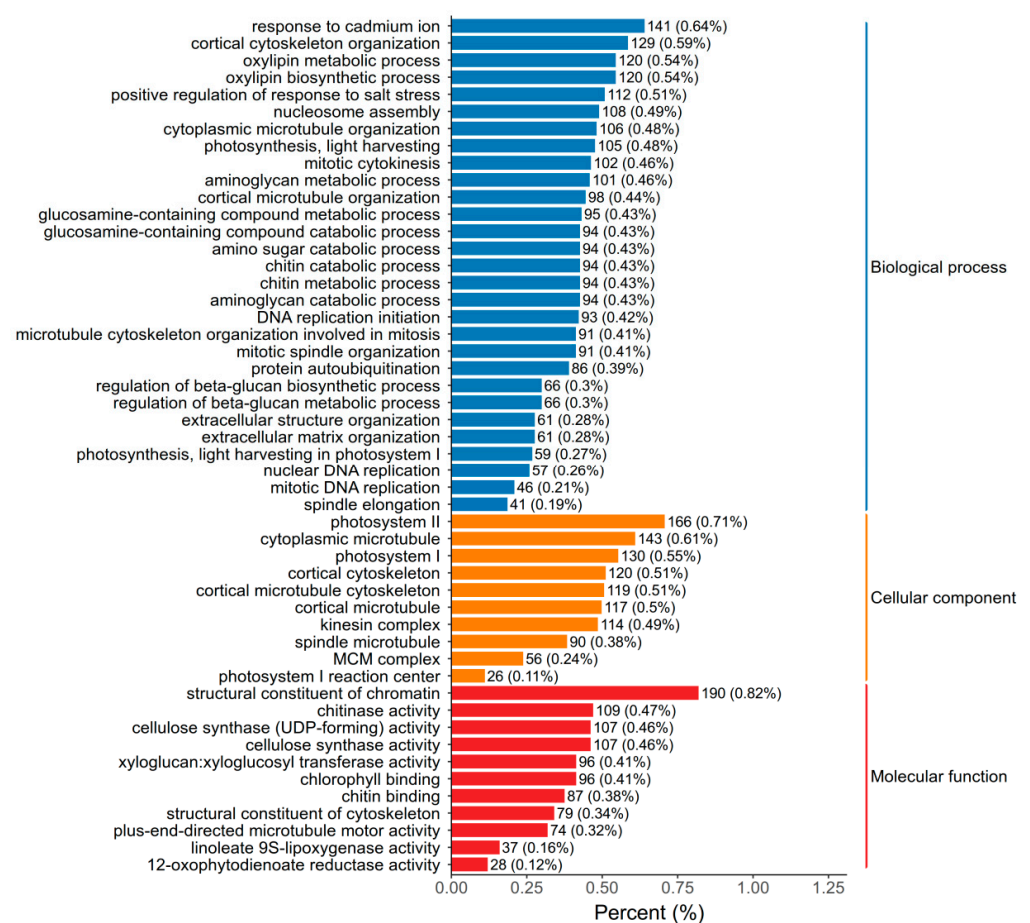


Figure 5. GO enrichment analysis of differentially expressed genes (DEGs). The horizontal axis denotes the proportion of DEGs assigned to each GO term, while the vertical axis enumerates the specific GO terms. For each term, the annotated gene count and its corresponding percentage of the total DEG set are displayed to the left of each bar.

Collectively, the GO enrichment results indicate that sugarcane's response to stem borer stress is a systematic biological process, involving cellular structure organization, basic metabolism, developmental regulation, stress signal transduction, and coordinated reprogramming of molecular functions. These findings provide important clues for further exploring the insect resistance mechanism of sugarcane.

3.1.5. KEGG Analysis of Differentially Expressed Genes

KEGG pathway enrichment analysis was performed to gain systematic insight into the biological functions of differentially expressed genes (DEGs) in sugarcane under borer herbivory (Figure 6). A total of 13,281 DEGs were assigned to 150 KEGG pathways, spanning five major categories. Notably, the “Metabolism” category contained the majority of annotated genes, underscoring a profound metabolic reprogramming in response to stress.

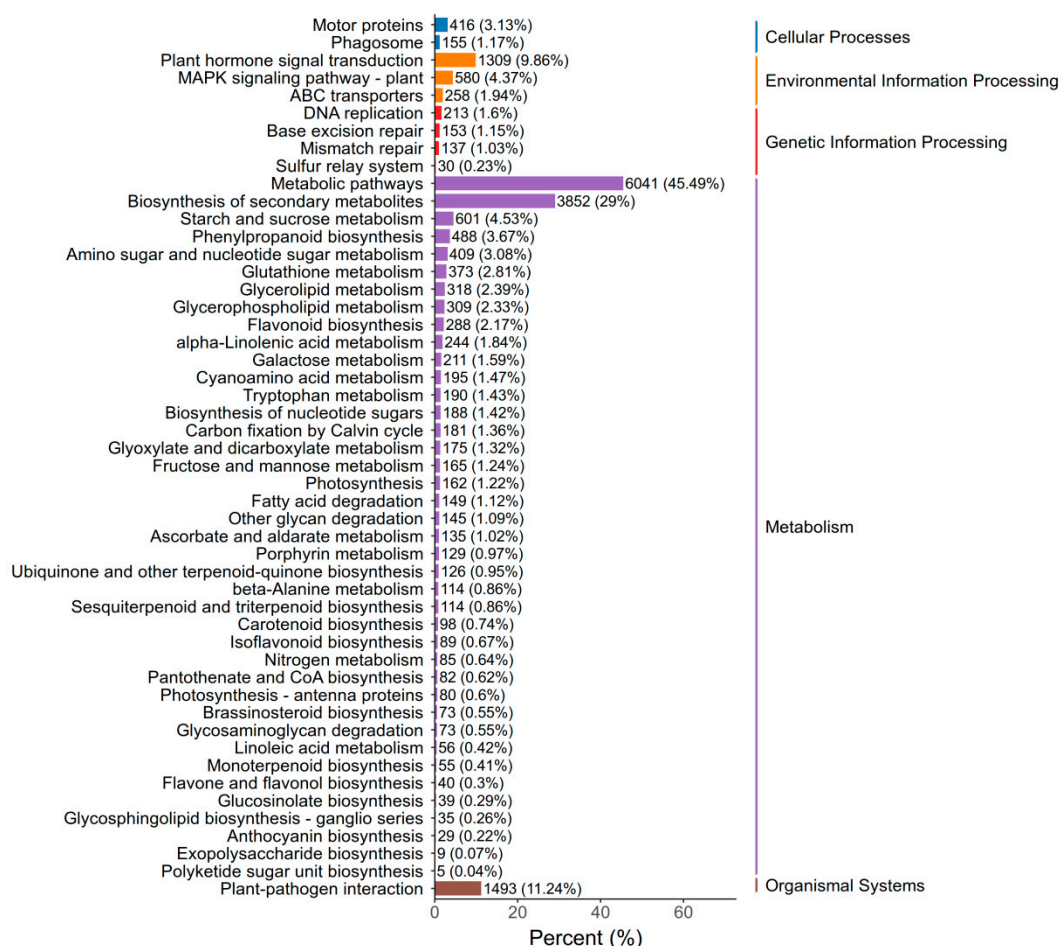


Figure 6. Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis of differentially expressed genes (DEGs). The horizontal axis indicates the percentage of DEGs mapped to each pathway, while the values to the left of each bar denote the number of DEGs and their corresponding percentage of the total DEG set.

Within “metabolism”, the most significantly represented pathways included “metabolic pathways” (45.49%) and “biosynthesis of secondary metabolites” (29%), highlighting a global shift in core and specialized metabolism. Key enriched sub-pathways involved in primary and secondary metabolism were identified (Figure 5). Of particular relevance to plant defense, pathways for “phenylpropanoid biosynthesis”, “flavonoid biosynthesis”, and “alpha-linolenic acid metabolism” (a precursor to jasmonate signaling) were significantly enriched. In “carbohydrate metabolism”, “starch and sucrose metabolism” was the most prominent.

Beyond metabolism, several key pathways in other categories were strongly enriched. Under “environmental information processing”, “plant hormone signal transduction” (9.86%) and the “MAPK signaling pathway—plant” (4.37%) were prominent, indicating active stress signaling. In “organismal systems”, the “plant-pathogen interaction” pathway (11.24%) was highly represented, suggesting a potential overlap between herbivore and

pathogen response mechanisms. Significant pathways in “genetic information processing” (e.g., “ubiquitin-mediated proteolysis”) and “cellular processes” (e.g., “endocytosis”) were also identified.

Collectively, the KEGG analysis reveals that the sugarcane transcriptomic response to stem borer is multifaceted, dominated by extensive metabolic restructuring, activation of stress-signaling cascades, and mobilization of defense-related secondary metabolite synthesis.

3.1.6. K-Means Co-Expression Clustering of Differentially Expressed Genes

To dissect the transcriptional response patterns of sugarcane DEGs under *C. sacchariphagus* natural infestation, K-means clustering grouped all DEGs into four distinct subclasses (Figure 7): Subclass 1 (13,560 DEGs) showed a significant up-regulation in Z4-T (infested Zhongtang 4), followed by a gradual decrease in non-infested and other cultivar samples, reflecting Z4-specific infestation-induced up-regulation. Subclass 2 (17,439 DEGs) exhibited high expression in Z4-T but was suppressed in non-infested groups, corresponding to stress-sensitive transcriptional responses. Subclass 3 (21,658 DEGs, the largest subclass) displayed a distinct expression trough in Z5-T (infested Zhongtang 5), indicating Z5-specific infestation-induced down-regulation (likely related to metabolic resource reallocation). Subclass 4 (15,428 DEGs) maintained stable expression in most samples but was significantly up-regulated in Z6-T (infested Zhongtang 6), representing Z6-specific defense-related transcriptional activation. These results fully reveal the cultivar-specific transcriptional reprogramming characteristics of sugarcane in response to borer infestation, laying a foundation for subsequent mining of cultivar-specific resistance genes.

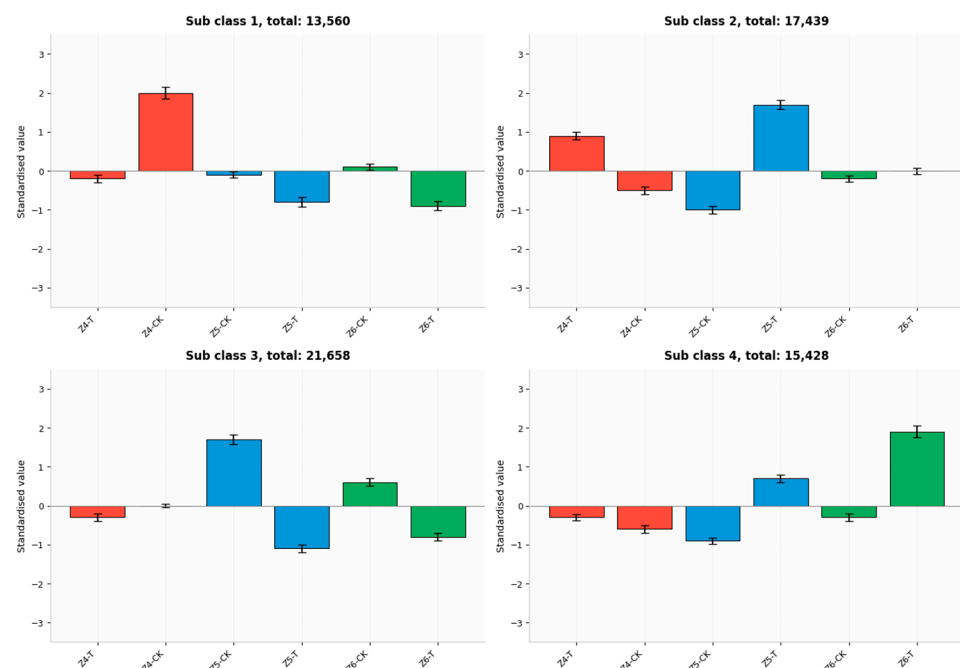


Figure 7. K-means co-expression clustering of differentially expressed genes in sugarcane cultivars Zhongtang 4, 5, and 6 under natural *C. sacchariphagus* infestation. The X-axis represents the sample groups, including T (infested) and CK (control) groups for lines Z4, Z5, and Z6. The Y-axis shows standardized gene expression values, indicating the relative expression levels within each subclass. The red, blue, and green color blocks represent varieties Z4, Z5, and Z6, respectively, with error bars indicating the variability in expression values within each group.

3.2. Metabolome Composition Analyses

3.2.1. Principal Component Analysis (PCA)

Principal component analysis (PCA) was performed to evaluate overall variation in the metabolomic profiles of three sugarcane cultivars (Zhongtang 4, 5, and 6) under both infestation by *C. sacchariphagus* and control conditions. The PCA score plot showed clear separation between infested (T) and control (CK) groups along the first principal component (PC1), which accounted for 37.26% of the total variance (Figure 8). This separation indicates that herbivory induced a consistent metabolic reprogramming across cultivars, generating a stress-related metabolomic signature distinct from that of undamaged plants.

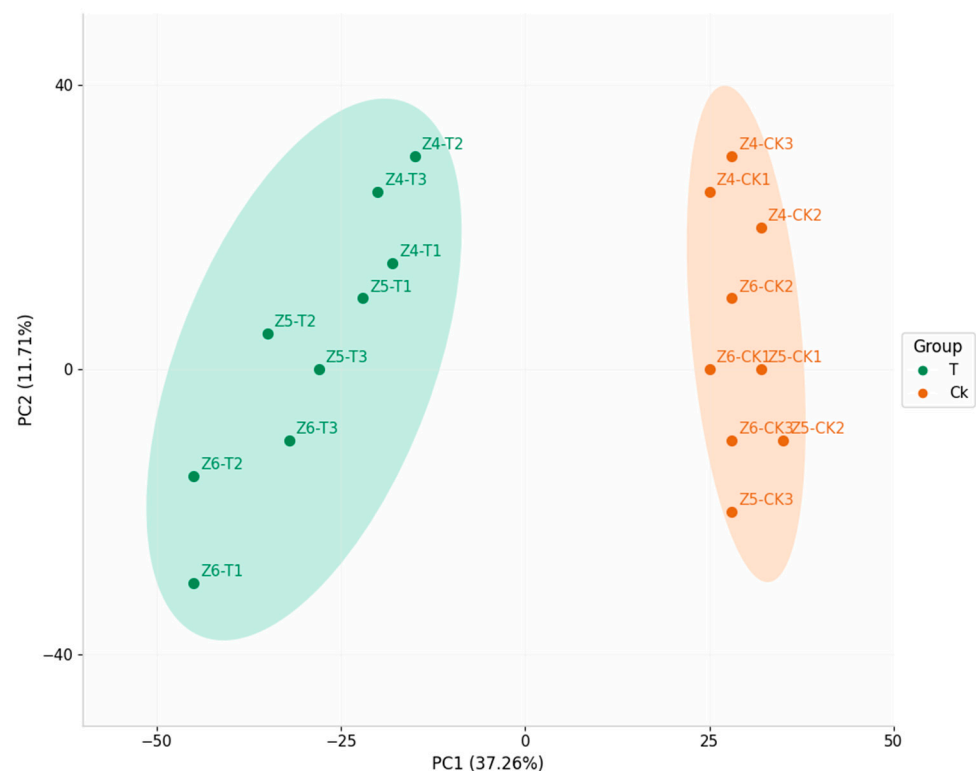


Figure 8. Principal component analysis (PCA) of metabolomic profiles showing separation between infested (T) and control (CK) groups across three sugarcane cultivars. Principal component analysis (PCA) of metabolomic data from three sugarcane cultivars (Zhongtang 4, 5, and 6) under *C. sacchariphagus* infestation (T, green) and control (CK, orange) conditions. PC1 (37.26%) and PC2 (11.71%) together explain 48.97% of the total metabolomic variance. The clear separation between infested and control groups along PC1 indicates that herbivory induces a consistent, distinct metabolic reprogramming across cultivars, while partial clustering of cultivars along PC2 suggests genotype-specific metabolic responses.

The second principal component (PC2, 11.71%) further separated samples based on cultivar identity, with Zhongtang 4, 5, and 6 showing partial clustering within both infested and control groups. This suggests that each cultivar possesses inherent, genotype-specific metabolic differences even under identical treatment conditions. Together, PC1 and PC2 explained 48.97% of the total metabolomic variance, highlighting the dominant effect of insect infestation on metabolic profiles while also revealing underlying genetic variation in both baseline and stress-induced metabolic responses.

These results demonstrate that *C. sacchariphagus* infestation drives a conserved yet cultivar-specific metabolic response in sugarcane, laying a foundation for identifying key metabolites and pathways associated with insect resistance.

3.2.2. Differential Metabolite Screening

Differential accumulated metabolites (DAMs) were identified based on criteria of variable importance in projection (VIP) > 1.0, absolute fold change ≥ 2 , and a p -value < 0.05 in the OPLS-DA model. This analysis identified 963 DAMs, comprising 683 up-regulated and 280 down-regulated metabolites (Figure 9). These metabolites were primarily classified into lipids, fatty acids, flavonoids, phenolic acids, alkaloids, terpenoids, amino acids, and their derivatives, indicating extensive metabolic reprogramming in sugarcane following borer stress.

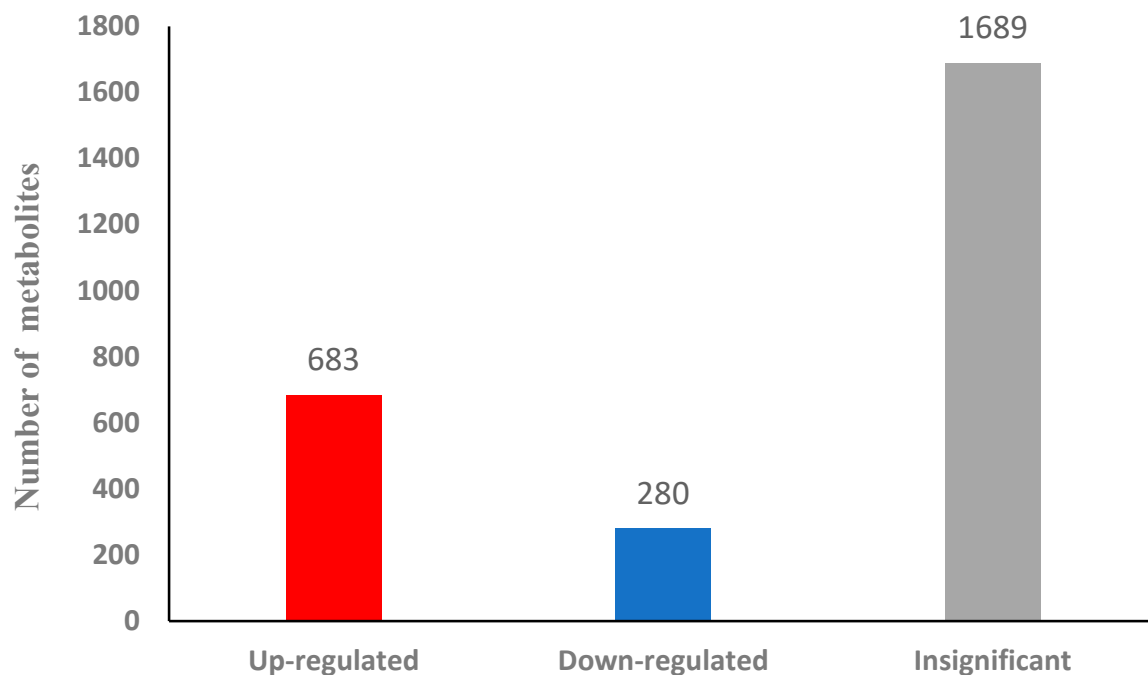


Figure 9. Distribution of metabolite expression patterns. Red indicates significantly up-regulated metabolites, blue indicates significantly down-regulated metabolites and gray indicates metabolites with no significant expression difference. The specific number of metabolites in each category is labeled on the top of the corresponding column.

The top 10 most significantly up-regulated metabolites are listed in Table 3. N-Acetyl-9-O-lactoylneuraminic acid exhibited the most pronounced accumulation (Fold Change = 283.4, Log_2FC = 8.15). Seryl-leucyl-valine (Fold Change = 146.1, Log_2FC = 7.19) and L-valyl-L-histidine (Fold Change = 141.0, Log_2FC = 7.14) were also markedly up-regulated. 2-Quinolinecarboxaldehyde showed the greatest down-regulation (Fold Change = 0.04, Log_2FC = −4.64, representing 4.0% of the control level), followed by methionine sulfoxide (Fold Change = 0.04, Log_2FC = −4.50) and (E,2Z)-2-[amino(carboxy)methylene]-5-oxopent-3-enoate (Fold Change = 0.05, Log_2FC = −4.22). These results demonstrate that the borer stress significantly perturbs metabolic processes in sugarcane, including the biosynthesis of sialic acid derivatives, the accumulation of peptides, and pathways related to quinoline alkaloid synthesis and methionine oxidation.

Table 3. Top 10 most significantly upregulated and downregulated differential metabolites in sugarcane varieties under sugarcane borer infestation compared with healthy control plants.

Index	Compounds	Class I	Fold_Change	Log ₂ FC	Type
MW0114941	N-Acetyl-9-O-lactoylneuraminic acid	Organic acids	283.45	8.15	up
MW0156832	Ser-Leu-Val	Amino acids and derivatives	146.15	7.19	up
MW0159267	Val-His	Amino acids and derivatives	141.03	7.14	up
MEDN1096	N-Benzoyl-L-tyrosine ethyl ester	Amino acids and derivatives	124.41	6.96	up
MW0003810	2-Amino-4-phenylphenol	Benzene and substituted derivatives	108.23	6.76	up
MW0010784	(+)-Isomenthone	Others	84.74	6.41	up
MW0108000	Lys-Glu-Lys	Amino acids and derivatives	83.61	6.39	up
MW0105857	Asp-Gly-Lys	Amino acids and derivatives	80.10	6.32	up
MW0156921	Ser-Thr-Asp	Amino acids and derivatives	78.69	6.30	up
MW0117404	Cyclopentanecarboxylic acid, 1-phenyl-, 2-piperidinoethyl ester	Others	69.33	6.12	up
MW0118886	2-Quinolinecarboxaldehyde	Heterocyclic compounds	0.04	−4.64	down
MEDP0060	Methionine sulfoxide	Amino acids and derivatives	0.04	−4.50	down
MW0168059	(E,Z)-2-[amino(carboxy)methylidene]-5-oxopent-3-enoate	Others	0.05	−4.22	down
MW0151218	HoPhe-Met-OH	Others	0.05	−4.21	down
MW0007593	Mescaline	Benzene and substituted derivatives	0.07	−3.85	down
MW0156675	Ser-Asp-Arg-Asp	Amino acids and derivatives	0.07	−3.79	down
MADN0532	L-Aspartyl-L-phenylalanine	Amino acids and derivatives	0.07	−3.77	down
MW0169166	Licoisoflavone A	Flavonoids	0.07	−3.77	down
MW0145563	Arg-Val-His	Amino acids and derivatives	0.08	−3.66	down
MW0124747	Mangiferin	Flavonoids	0.08	−3.59	down

Note: This table lists the top 10 upregulated and downregulated metabolites, prioritized by absolute log₂ fold change and statistical significance. Index: Unique identifier for each metabolite. Compounds: Name of the differential metabolite. Class I: Primary chemical classification of the metabolite. Fold Change: Ratio of metabolite abundance in borer-infested plants to that in healthy control plants. Log₂FC: Log₂-transformed fold change; positive values indicate increased abundance, and negative values indicate decreased abundance. Type: Direction of differential abundance (up = upregulated; down = downregulated).

3.2.3. KEGG Pathway Enrichment Analysis of Differential Metabolites

KEGG enrichment analysis revealed that the differentially accumulated metabolites (DAMs) were significantly involved in 82 distinct pathways. Of the 406 identified metabolites, 151 were successfully annotated to these pathways (Figure 10). The most prominently enriched pathways included (1) amino acid metabolism (e.g., phenylalanine, tyrosine and tryptophan biosynthesis), (2) biosynthesis of secondary metabolites (e.g., phenylpropanoid, flavone and flavonol biosynthesis), and (3) metabolism of co-factors and vitamins. Pathways for glutathione metabolism and ABC transporters were also notably enriched.

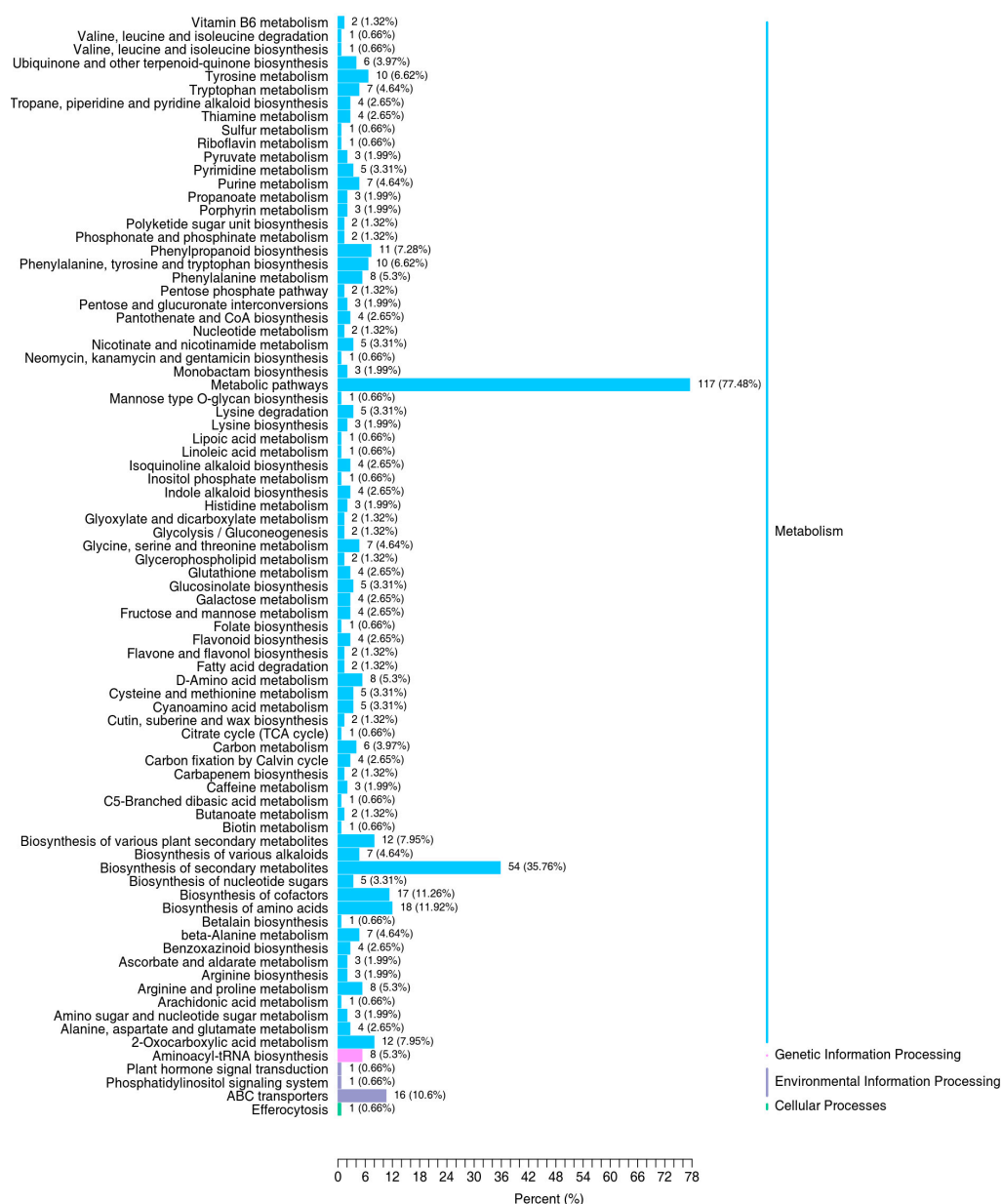


Figure 10. KEGG classification chart of differential metabolites. The Y-axis indicates significantly enriched metabolic pathways. The X-axis represents the number of differential metabolites mapped to each pathway and their percentage relative to the total annotated differential metabolites.

A systematic analysis of these pathways highlighted the global metabolic impact of herbivory (Figure 11). The phenylpropanoid biosynthesis pathway, tightly linked to plant stress resistance, was strongly activated and served as a key biosynthetic route for defensive compounds, such as lignin and flavonoids. Concurrently, other defense-related pathways (e.g., benzoxazinoid, indole alkaloid, and glucosinolate biosynthesis) were significantly enriched, indicating a multi-layered chemical defense response. Extensive reprogramming of primary metabolism was also observed. Enrichment in pathways for amino acid biosynthesis and aminoacyl-tRNA biosynthesis suggested enhanced protein synthesis and turnover. In energy metabolism, the activation of fatty acid degradation and ubiquinone biosynthesis indicated mobilization of energy reserves and support for mitochondrial respiration. Significant enrichment of glutathione metabolism further implied oxidative stress and antioxidant system activation.

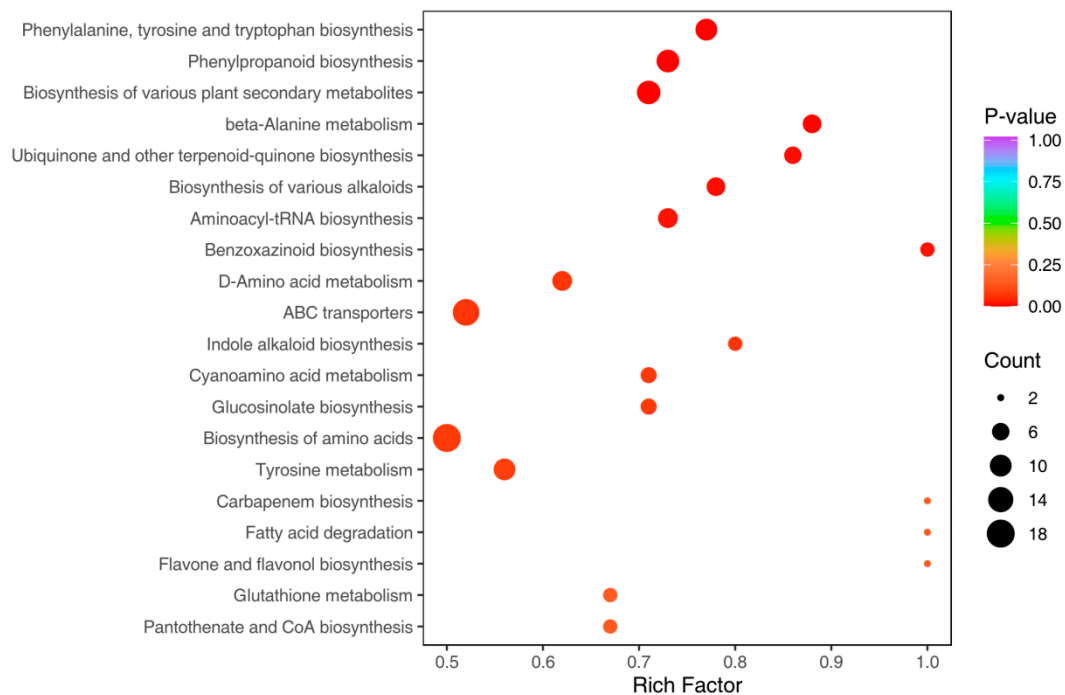


Figure 11. Scatter plot of enriched KEGG pathways for differentially accumulated metabolites (DAMs). The y-axis displays pathway names, ordered by enrichment significance (p -value). The x-axis represents the Rich Factor for each pathway. The color of the data points corresponds to the p -value, with a redder hue indicating a more significant enrichment. The size of the points reflects the number of DAMs enriched in the corresponding pathway.

In summary, borer infestation induced not only the biosynthesis of defense-related secondary metabolites but also a comprehensive restructuring of primary and energy metabolism, collectively shaping sugarcane's integrated physiological response.

3.2.4. K-Means Clustering of Metabolomic Profiles Reveals Distinct Response Patterns to Insect Infestation

To characterize the global metabolic changes induced by *C. sacchariphagus* infestation in different sugarcane cultivars, K-means clustering analysis was performed on the standardized abundance of all detected metabolites. The metabolites were divided into two major subclasses based on their accumulation patterns (Figure 12):

Subclass 1 (774 metabolites) showed a coordinated up-regulation pattern, with significantly increased abundance specifically in infested samples of Z4 and Z6 (Z4-T, Z6-T) and relatively stable levels in Z5-CK. This pattern suggests that metabolites in Subclass 1 are strongly and positively responsive to insect herbivory in a cultivar-dependent manner, potentially representing core induced defense-related compounds.

Subclass 2 (1187 metabolites) exhibited a distinct down-regulation pattern, with significantly decreased abundance specifically in infested Z5 (Z5-T) compared to other samples. This suppression indicates that more metabolic pathways may be actively downregulated in certain genetic backgrounds in response to infestation, possibly reflecting reallocation of biochemical resources or cultivar-specific susceptibility traits.

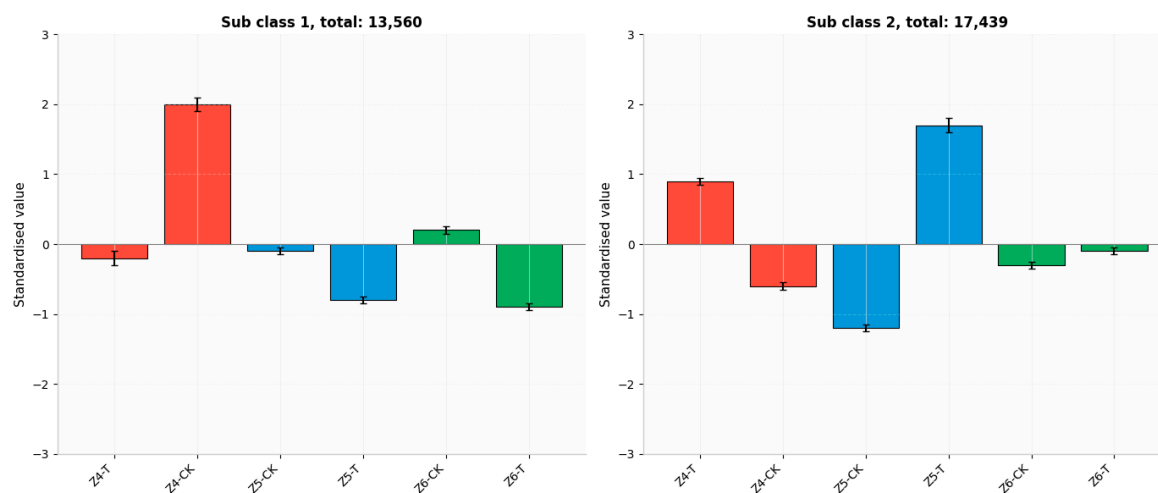


Figure 12. K-means clustering reveals cultivar-specific metabolic responses to insect herbivory. The X-axis denotes sample groups, including infested (T) and control (CK) treatments for each cultivar (Z4, Z5, and Z6). The Y-axis shows standardized gene expression values, representing relative expression levels across treatments. The color blocks (red, blue, and green) correspond to cultivars Z4, Z5, and Z6, respectively, and error bars indicate within-group variability.

This clear separation of metabolites into two opposing response clusters highlights the complexity and cultivar-specificity of metabolic reprogramming during insect attack. The coordinated up-regulation in Z4 and Z6 (Subclass 1) vs. the predominant down-regulation in Z5 (Subclass 2) aligns with the transcriptional strategies, suggesting Z4 and Z6 may actively accumulate defense compounds, while Z5 might employ a resource reallocation or susceptibility-related strategy.

3.3. Integrated Analysis of Transcriptome and Metabolome

3.3.1. Expression Correlation Analysis

To elucidate the systemic molecular response of sugarcane stems to natural infestation by *C. sacchariphagus*, a joint analysis of transcriptomic and metabolomic data was performed. The global correlation between differentially expressed genes (DEGs) and differentially accumulated metabolites (DAMs) was visualized using a nine-quadrant plot (Figure 13). The distribution of gene-metabolite association pairs across the quadrants revealed distinct regulatory patterns. A significant number of pairs clustered in Quadrant I (both genes and metabolites significantly upregulated) and Quadrant IX (both genes and metabolites significantly downregulated), indicating concerted transcriptional and metabolic reprogramming in response to insect infestation. These co-upregulated pairs in Quadrant I are of particular interest, as they likely represent key defense-related pathways activated by the plant. Conversely, pairs located in Quadrant III (genes down, metabolites up) and Quadrant VII (genes up, metabolites down) suggest the presence of more complex, potentially non-linear regulatory mechanisms, such as post-translational regulation, feedback inhibition, or rapid metabolic turnover.

Transcriptome-metabolome association analysis revealed significant correlations between multiple genes and metabolites ($|r| > 0.98$, $p < 0.01$) (Table 4). The network exhibited characteristics of high interconnectivity and distinct bidirectional regulation. Known functional genes (e.g., Soffic.06G0009030-I-P) showed strong positive correlations with defense-related metabolites, such as fatty acid derivatives and phenolic acids (r up to 0.9931), directly promoting the biosynthesis of insect-resistant secondary metabolites. In contrast, novel transcripts (e.g., novel.19736) displayed significant negative correlations with various metabolites (r as low as -0.9796). Among these, novel.19736, acting as the

strongest negative regulatory hub, may mediate metabolic reprogramming through protein degradation inhibition pathways. The key metabolic hub, D-xylulose-5-phosphate, was subject to intense bidirectional positive and negative regulation, precisely balancing resource allocation between energy supply and defense synthesis under stress. This study identified multiple super-hub nodes, including the global negative regulator novel.19313, and found that over half of the core regulatory genes were novel genes with unknown functions. These findings provide new critical targets and research directions for elucidating the molecular network underlying plant insect resistance.

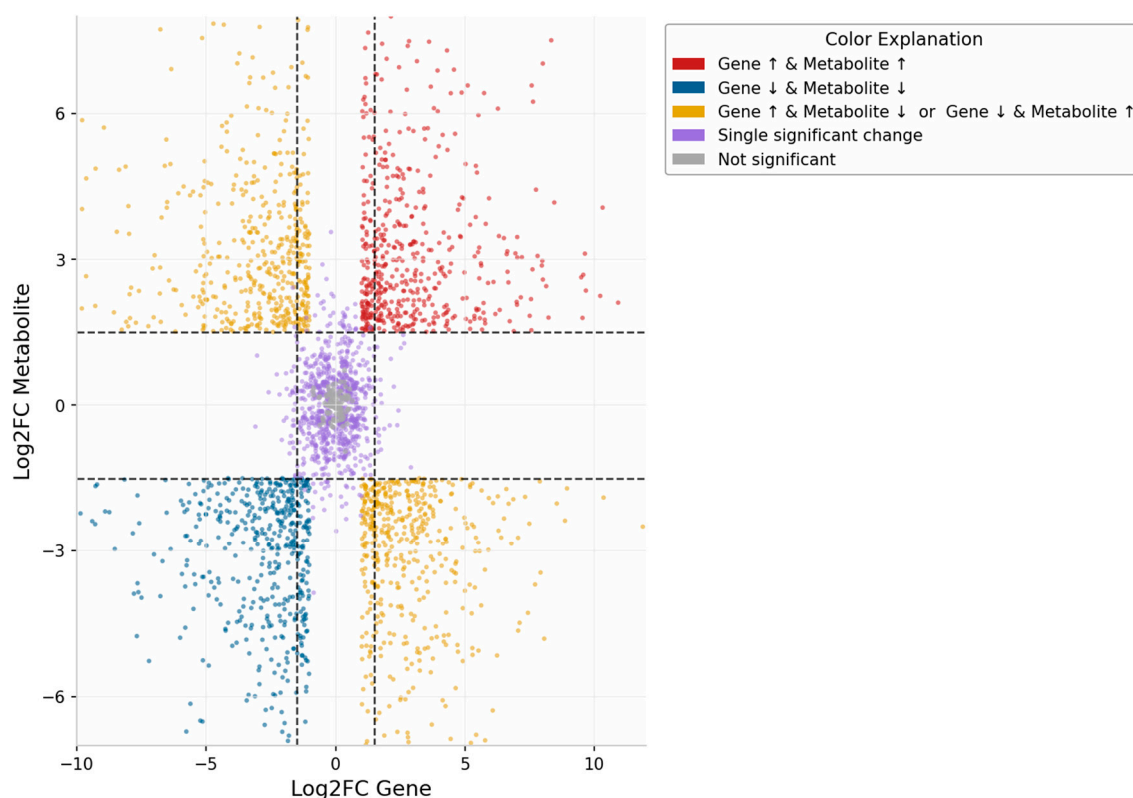


Figure 13. Correlation analysis nine-quadrant plot. Note. Each point represents a pair of correlations, with the x-axis denoting the gene's Log₂FC and the y-axis denoting the metabolite's Log₂FC.

Table 4. Top 10 gene-metabolite associations with the strongest positive and negative correlations in sugarcane under sugarcane borer infestation.

No.	Gene ID	KO	Metabolites	Class	r	p-Value
1	Soffic.06G0009030-1P	--	17-keto-DPA/17-Oxo-DPA	Fatty acids and derivatives	0.9931	2.53×10^{-16}
2	Soffic.10G0019290-2D	K01246	3,4-Dihydroxy-6H-benzo[c]chromen-6-one	Benzene derivatives	0.9926	4.36×10^{-16}
3	Soffic.07G0012580-1P	K01904	4-Isopropylbenzyl alcohol	Alcohol and amines	0.9922	6.89×10^{-16}
4	Soffic.02G0013930-1P	K13415	10-Gingerol	Phenolic acids	0.9915	1.28×10^{-15}
5	Soffic.09G0016310-3C	K20729	D-Xylulose 5-phosphate	Others	0.9902	4.06×10^{-15}
6	Soffic.09G0005010-6H	K15803	4-Isopropylbenzyl alcohol	Alcohol and amines	0.9902	4.08×10^{-15}
7	Soffic.05G0013050-2G	K08081	N- α -Acetyllysine	Amino acids derivatives	0.9902	4.28×10^{-15}
8	Soffic.01G0040950-6G	K12811	Avocadyne	Others	0.9899	5.11×10^{-15}
9	Soffic.01G0040520-1T	K16296	N,N-Dimethyl-L-valine	Amino acids derivatives	0.9894	7.78×10^{-15}
10	Soffic.01G0023340-1P	--	4,7-Dimethyl-1-tetralone	Benzene derivatives	0.9893	8.29×10^{-15}
11	novel.19736	K07466	N- α -Acetyllysine	Amino acids derivatives	-0.9894	7.65×10^{-15}

Table 4. Cont.

No.	Gene ID	KO	Metabolites	Class	r	p-Value
12	novel.19736	K07466	Allyl methyl sulfoxide	Others	−0.9853	1.02×10^{-13}
13	novel.19736	K07466	Vasicinol	Alcohol and amines	−0.983	3.29×10^{-13}
14	novel.19736	K07466	L-2-Amino-3-(1-pyrazolyl)propanoic acid	Organic acids	−0.9822	4.74×10^{-13}
15	Soffic.01G0030010-1A	K19995	Tolbutamide	Benzene derivatives	−0.9805	9.90×10^{-13}
16	novel.19736	K07466	Vanillylamine	Alkaloids	−0.9804	1.01×10^{-12}
17	novel.24609	K00850	α-Amylcinnamaldehyde	Others	−0.9801	1.18×10^{-12}
18	Soffic.01G0030010-1A	K19995	D-Xylulose 5-phosphate	Others	−0.9796	1.41×10^{-12}
19	novel.22655	K00521	α-Amylcinnamaldehyde	Others	−0.9794	1.55×10^{-12}
20	novel.22655	K00521	3,5-Dihydroxy-1,4-naphthoquinone	Benzene derivatives	−0.9792	1.67×10^{-12}

Note: This table presents the top 10 positively and negatively correlated gene-metabolite pairs, identified by Pearson correlation analysis of transcriptomic and metabolomic data from borer-infested sugarcane. Gene ID: Unique identifier of the differentially expressed gene. KO: Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology identifier for the gene; -- indicates no KO annotation available. metabolites: Name of the differentially abundant metabolite. Class: Chemical classification of the metabolite. r: Pearson correlation coefficient (ranging from −1 to 1; values close to 1 indicate strong positive correlation, values close to −1 indicate strong negative correlation). p-value: Statistical significance of the correlation; all p-values < 0.001, indicating highly significant associations.

3.3.2. The KEGG Analysis

To understand the metabolic reprogramming induced by insect herbivory at the system level, we performed joint KEGG pathway enrichment analysis on differentially expressed genes (DEGs) and differentially accumulated metabolites (DAMs) between infested and control sugarcane plants and analyzed the transcriptome gene distribution across metabolic pathways (Figure 14) and the enrichment characteristics of metabolic pathways in both omics datasets (Figure 15).

Transcriptome gene distribution analysis showed that core metabolic pathways (e.g., Metabolic pathways, ~6000 genes) and secondary metabolite biosynthesis pathways (~4000 genes) contained the most transcriptome genes, while specific metabolic pathways (e.g., Vitamin B6 metabolism) had very few (close to zero), indicating that fundamental metabolic pathways are subject to larger-scale transcriptional regulation (Figure 14).

The enrichment analysis revealed significant rewiring of primary and specialized metabolism. Core pathways (e.g., Metabolic pathways, secondary metabolite biosynthesis) had low enrichment factors (<0.25) but p-values close to zero, showing extremely significant enrichment. In contrast, some secondary metabolic pathways (e.g., Flavone and flavonol biosynthesis) had higher enrichment factors (>0.75) but higher p-values (>0.25), with weaker enrichment significance (Figure 15).

Notably, secondary metabolite biosynthesis and phenylpropanoid/flavonoid biosynthesis pathways were highly enriched with numerous DEGs and corresponding DAMs, indicating strong activation of chemical defense-related compound production. Meanwhile, key primary metabolic pathways (e.g., glycolysis/gluconeogenesis, carbon fixation, and amino acid metabolism) were significantly altered, suggesting reallocation of carbon and nitrogen resources from growth to defense precursor and antioxidant synthesis.

Additionally, plant hormone signal transduction, lipid metabolism (e.g., glycerophospholipid metabolism), and sugar/nucleotide metabolism pathways were perturbed, reflecting comprehensive changes in defense signaling, membrane dynamics, energy supply, and biosynthesis capacity under stress.

Convergent enrichment patterns in the two omics layers indicate that sugarcane responds to *C. sacchariphagus* infestation by coordinately upregulating defensive secondary metabolite production and restructuring central metabolic fluxes to support defense mobilization.

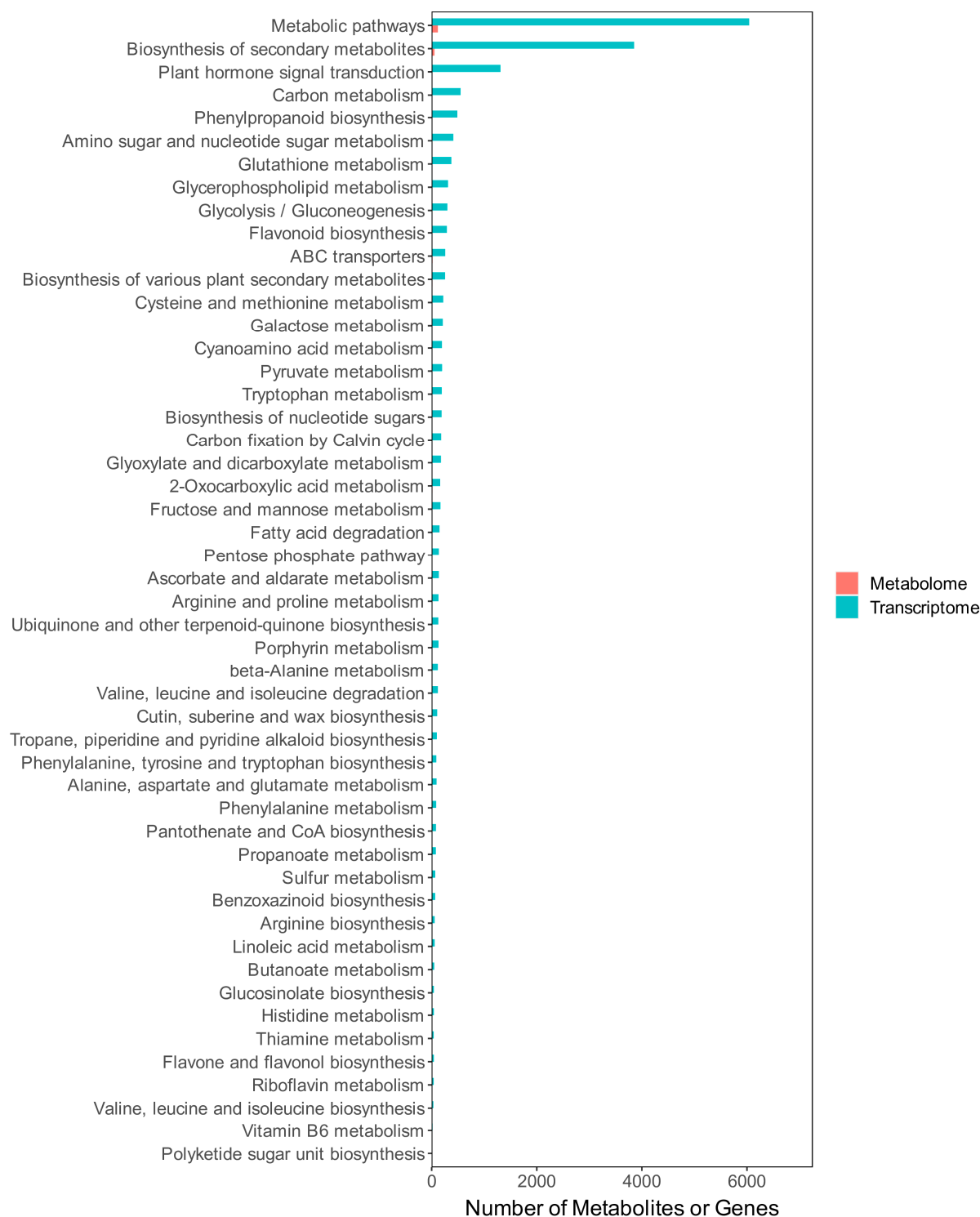


Figure 14. Enrichment of metabolic pathways for differentially accumulated metabolites and differentially expressed genes in *Saccharum* spp. (sugarcane) under *C. sacchariphagus* (sugarcane borer) infestation. X-axis: Count of metabolites/genes assigned to each pathway. Y-axis: Annotated metabolic pathways (KEGG database). Color coding: Red = Number of differentially accumulated metabolites; Green = Number of differentially expressed genes. Pathways with high metabolite/gene counts (e.g., Metabolic pathways, Biosynthesis of secondary metabolites) represent key metabolic and transcriptional reprogramming events in sugarcane during *C. sacchariphagus* infestation.

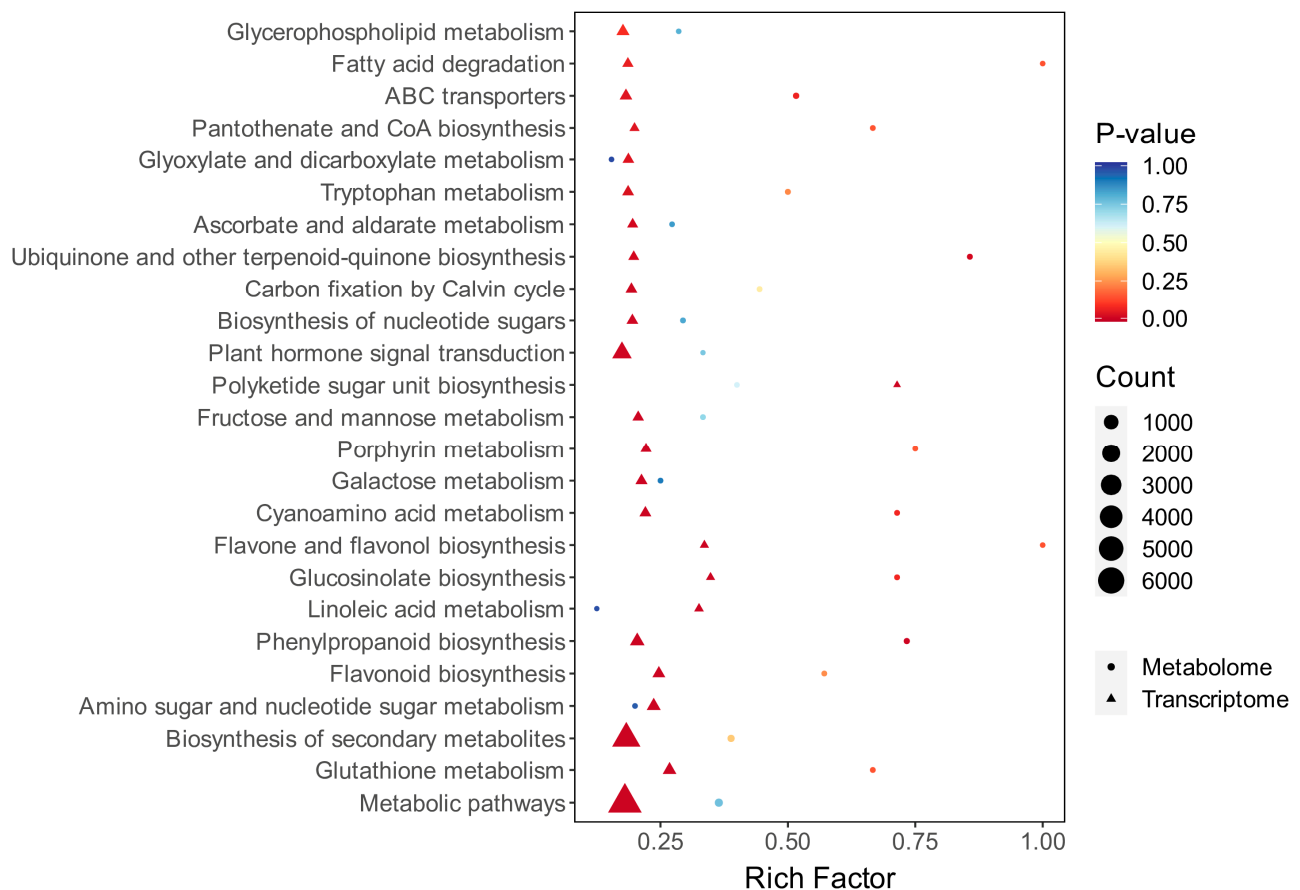


Figure 15. The KEGG enrichment analysis bubble plot. The x-axis represents the enrichment factor (Diff/Background) for the pathways across different omics datasets. The y-axis displays the corresponding KEGG pathway names. A color gradient (red-yellow-blue) is used to visualize the level of significance based on p -value, with red indicating the highest significance. The shape of the bubbles corresponds to the different omics types, while the bubble size is proportional to the number of differential metabolites or genes.

3.3.3. Co-Expression Clustering Analysis

To investigate transcriptional and metabolic reprogramming in sugarcane after *C. sacchariphagus* infestation, we performed K-means co-expression clustering on transcriptomic and metabolomic data acquired in negative and positive ionization modes. Six consistent co-expression clusters were identified, each showing distinct variety-specific patterns.

Under negative ionization (Figure 16), 53,064 genes and 2652 metabolites were grouped into six clusters. Cluster 1—the largest with 17,016 genes and 670 metabolites—was significantly enriched in infested Z4 (Z4-T) vs. control (Z4-CK), suggesting Z4-specific defense activation. Cluster 6 (13,069 genes, 682 metabolites) showed multi-variety enrichment, indicating conserved stress responses. Clusters 2 and 4 were predominantly associated with Z5, highlighting variety-specific regulation.

Under positive ionization (Figure 17), clustering patterns differed markedly. Total genes increased to 82,960, with Cluster 6 becoming hyper-enriched (30,311 genes, 36.5% of total; 682 metabolites) and co-enriched in Z5 and Z6. Cluster 2 (11,807 genes) was enriched mainly in Z5, Cluster 5 (9509 genes) in Z6, and Cluster 3 (14,055 genes) in Z4.

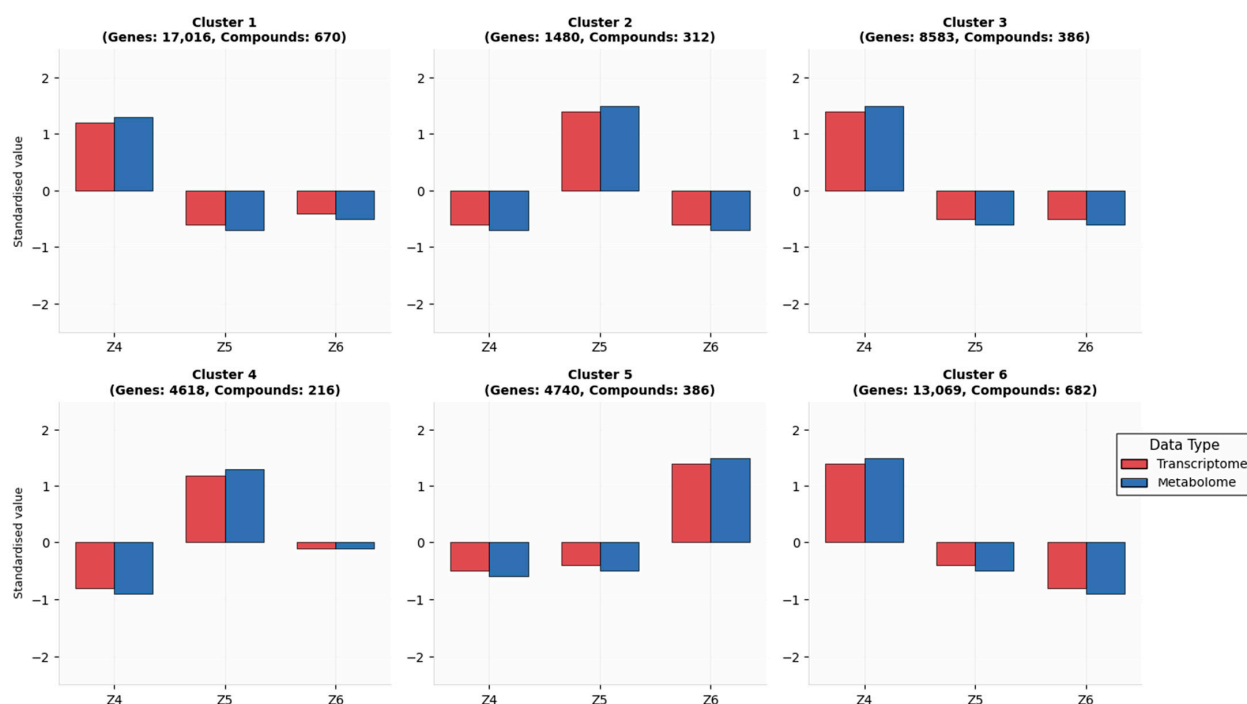


Figure 16. K-means co-expression cluster plot of integrated transcriptomic and metabolomic data in sugarcane under *C. sacchariphagus* Infestation (Negative Regulation Module). The total number of genes and annotated metabolites within each cluster is indicated in the parentheses of the subplot titles. The x-axis represents the three cultivars, while the y-axis displays standardized expression/abundance values, reflecting relative transcriptional and metabolic changes. Transcriptomic data are shown in red, and metabolomic data in blue, with the height of each block corresponding to the magnitude of the standardized value.

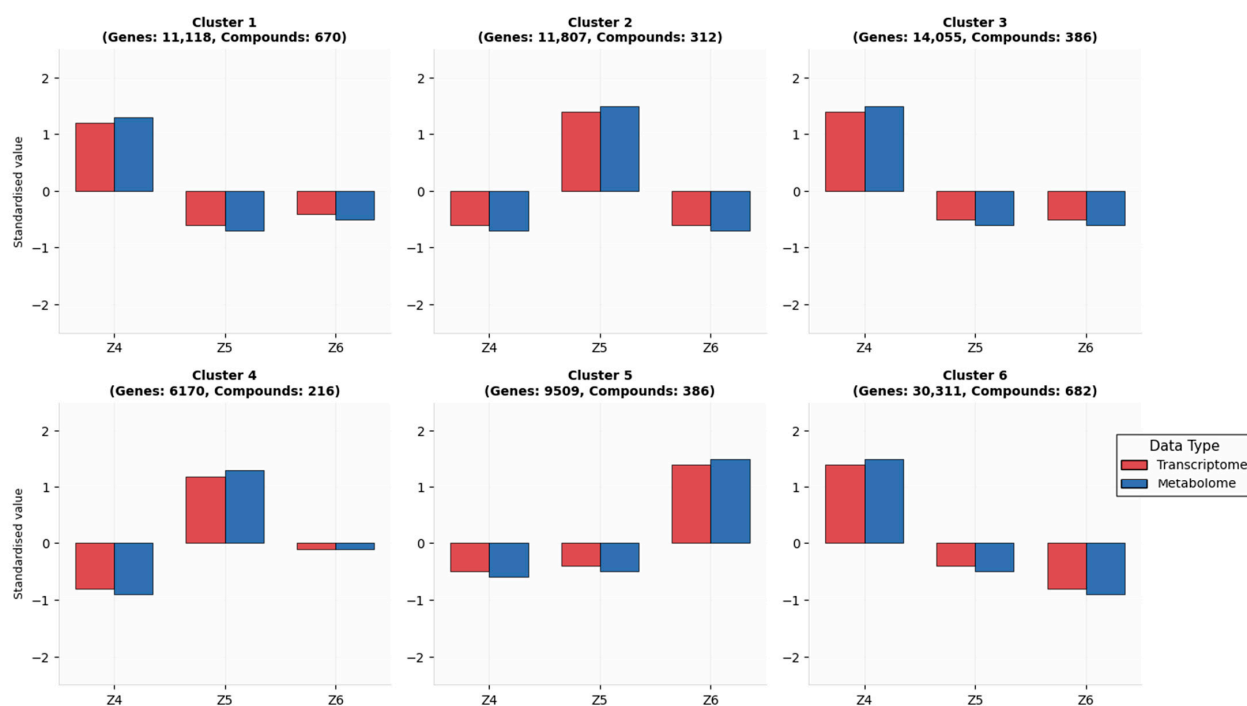


Figure 17. K-means co-expression cluster plot of integrated transcriptomic and metabolomic data in sugarcane under *C. sacchariphagus* Infestation (Positive Regulation Module). The total number of genes

and annotated metabolites within each cluster is indicated in the parentheses of the subplot titles. The x-axis represents the three cultivars, while the y-axis displays standardized expression/abundance values, reflecting relative transcriptional and metabolic changes. Transcriptomic data are shown in red, and metabolomic data in blue, with the height of each block corresponding to the magnitude of the standardized value.

Notably, transcriptional and metabolic profiles within each cluster were strongly concordant across varieties and ionization modes, reflecting synchronized reprogramming induced by borer infestation.

Together, these results indicate variety-dependent defense strategies. Z4 relies primarily on pathways captured in negative mode (Clusters 1 and 3), whereas Z5 and Z6 activate a large, coordinated response evident in positive mode (Cluster 6). The distinct clustering patterns—particularly the Z4-specific Cluster 1 (negative mode) and the conserved mega-Cluster 6 (positive mode)—provide key insights into the molecular mechanisms underlying variety-specific resistance.

4. Discussion

The intricate inducible defense system of plants, refined through co-evolution with herbivores, enables rapid and coordinated reprogramming at molecular and biochemical levels upon attack [59–63]. Our integrated transcriptomic and metabolomic analysis provides a comprehensive, systems-level perspective on how sugarcane (*Saccharum* spp.) mobilizes its defenses against the borer *C. sacchariphagus*. The massive scale of the response—with 34,004 differentially expressed genes (DEGs) and 963 differentially accumulated metabolites (DAMs)—underscores that borer infestation triggers not isolated reactions but a profound systemic physiological overhaul. Critically, this study elucidates that different sugarcane cultivars have evolved three distinct molecular response strategies to the same pest challenge: the “suppressive-response type” (global transcriptional repression) represented by Z4, the “activating-response type” (aggressive transcriptional activation) represented by Z5, and the “balanced-response type” (fine-tuned mixed regulation) represented by Z6. These patterns, revealed by gene expression clustering and K-means co-expression analysis, indicate that the genetic background dictates the core transcriptional strategy, manifesting as distinct pathways for coping with borer stress. These findings reveal both conserved defense mechanisms shared across plants and unique adaptive strategies specific to sugarcane, providing a molecular basis for dissecting the genetic basis of borer resistance in this crop.

4.1. Global Transcriptional Reprogramming: The Foundation of Systemic Defense

Transcriptome sequencing confirmed data reliability, and the identification of 34,004 DEGs (54.92% up-regulated) indicated extensive transcriptional reprogramming. This sheer number of DEGs, significantly enriched in broad GO categories like “cellular process”, “metabolic process”, and “response to stimulus”, signifies a wholesale shift in sugarcane’s biological priorities from growth to defense, a classic resource trade-off. The KEGG analysis further highlighted that this reprogramming was systematic, with dominant enrichment in “metabolic pathways” and “biosynthesis of secondary metabolites”, alongside key signaling pathways like “plant hormone signal transduction” and “plant–pathogen interaction”. This extensive activation across numerous functional hierarchies vividly embodies the dynamic and systemic core characteristics of plant-induced resistance [64]. The high enrichment of the “plant–pathogen Interaction” pathway indicates that sugarcane may employ or exchange components of disease resistance mechanisms during its defense against insects. This is further illustrated in rice, where defense against the brown planthopper involves the pathogen-responsive OsPROPEP3/OsPEPRs pathway

and phenylpropanoid metabolism [65]. This observation is consistent with findings in other systems, such as the hypersensitive response (HR) and intense metabolic reprogramming involving over 7000 DEGs and 80 differential metabolites noted during incompatible rice-gall midge interactions [66], and the activation of defense-related phytohormone and secondary metabolic pathways alongside growth suppression in rice responding to *C. suppressalis* infestation [34]. These parallels imply extensive cross-talk and synergistic integration within plant defense signaling networks against diverse biotic stresses, as shaped by complex hormonal interactions [67], which may represent a crucial strategy for achieving broad-spectrum resistance.

The observation that different sugarcane cultivars employ distinct molecular strategies—global suppression in Z4, aggressive activation in Z5, and balanced regulation in Z6—echoes the genotype-dependent defense responses documented in other plants, such as sugarcane-pathogen interactions [29] and rice-pest systems [28]. However, our study advances this understanding by demonstrating that these strategies are underpinned by coherent multi-omics reprogramming. K-means clustering revealed that the cultivar-specific transcriptional patterns (e.g., Subclass 1 in Z4, Subclass 3 in Z5, Subclass 4 in Z6) are tightly coupled with corresponding shifts in metabolite accumulation profiles (Section 3.2.4). This synergy indicates that the defense architecture of each cultivar is a system-wide property, integrating gene regulation with metabolic output. Consequently, the resistance mechanisms in Z4 and Z6 appear to be driven by the active co-induction of specific gene clusters and defense metabolites, whereas Z5's strategy may prioritize resource reallocation through coordinated down-regulation. This integrated view confirms that cultivar-specific adaptation to biotic stress is a common but complex phenomenon, best understood through a multi-omics lens.

4.2. The Metabolic Arsenal for Direct Defense: Specific Activation of Core Pathways

Direct defense relies on the synthesis of toxic or antinutritive compounds. The transcriptomic and metabolomic data from this study consistently indicate that the “phenylpropanoid biosynthesis” and “flavonoid (flavonol) biosynthesis” pathways are the central responsive hubs for direct defense in sugarcane challenged by *C. sacchariphagus*. This finding is consistent with observations across diverse plant-insect systems, underscoring the hypothesis that phenylpropanoid/flavonoid metabolism represents an evolutionarily conserved, core chemical foundation for insect resistance shared across both monocot and dicot plants. For instance, Liu et al. (2024) [68] demonstrated significant activation of phenylpropanoid and flavonoid biosynthesis in aphid-stressed alfalfa, while Zhang et al. (2021) [69] reported flavonoid and isoflavonoid biosynthesis as the most significantly enriched pathways in thrips-infested alfalfa. Similarly, Niu et al. (2018) [70] identified that Rm3 gene-mediated aphid resistance in peach relies on rapid induction of secondary metabolites from phenylpropanoid/flavonoid pathways. In soybean leaves, herbivory by *Spodoptera litura* and its oral secretions specifically induce the de novo biosynthesis and accumulation of flavone and isoflavone aglycones, a distinct defense response against chewing insects [71]. Similarly, in maize, herbivory by *Spodoptera exigua* co-upregulated the phenylpropanoid and benzoxazinoid pathways, with benzoxazinoid accumulation occurring via a potentially JA/SA-independent mechanism [72]. The products in question fulfil pivotal roles, and lignin serves to reinforce physical barriers, while flavonoids and phenolic acids exert direct antifeedant or toxic effects.

Our study delineates the cultivar-specific “chemical recipe” of sugarcane defense against stem borer at three levels. First, at the pathway level, we confirm common phenylpropanoid/flavonoid induction and identify the activation of more specialized routes (e.g., Indole Alkaloid biosynthesis), contrasting with other systems [73] but aligning with sugarcane-specific defenses [74]. Second, at the compound level, metabolomic screen-

ing pinpointed concrete candidates like the up-regulated peptide Ser-Leu-Val and the down-regulated flavonoid mangiferin (Figure S1). Most importantly, at the systems level, K-means clustering revealed that these metabolic changes are not random but form coherent, cultivar-specific programs. The active accumulation of Subclass 1 metabolites in Z4 and Z6 contrasts sharply with the broad suppression of Subclass 2 metabolites in Z5, mirroring transcriptional strategies and suggesting fundamentally different defense philosophies. While the anti-herbivore activity of each candidate awaits functional validation [30], our integrated analysis does more than list correlations. It embeds specific metabolites within a robust, multi-omics framework of cultivar-specific defense strategies, thereby transforming a catalog of chemical changes into a prioritized blueprint for mechanistic inquiry.

4.3. Metabolic Preparation and Energetic Support for Indirect Defense

The function of indirect defense is to release volatiles, which attract natural enemies. The multi-omics data provided offer a solid metabolic blueprint for this process. The significant enrichment of the “ α -Linolenic Acid Metabolism” (the lipoxygenase pathway) serves as a key marker for jasmonic acid (JA) biosynthesis, and the initiation of downstream volatile signaling, a pathway whose activation can be further fine-tuned by specific JA-amino acid conjugates to amplify the defense signal [75]. Concurrently, the activation of the “Terpenoid Backbone Biosynthesis” pathway supplies precursors for the synthesis of common herbivore-induced volatiles, such as monoterpenes and sesquiterpenes. These findings corroborate the observations by He et al. (2020) [73] of increased herbivore-induced terpenoid volatile emissions in cucumber to attract natural enemies, strongly suggesting that sugarcane initiates metabolic preparations for indirect defense upon borer herbivory. Furthermore, as shown by Wang et al. (2021) [76] in sugarcane under *Mythimna separata* herbivory, such metabolic shifts can lead to the accumulation of specific defense compounds like chlorogenic acid, which exhibits lethal effects against the pest, demonstrating the synergy between direct and indirect defense metabolism.

In addition, the process of sugarcane photosynthesis is characterized by the simultaneous enhancement of “fatty acid degradation” and “carbohydrate metabolism”. This phenomenon of metabolic flux channeling towards defense responses aligns with the concept proposed by Liu et al. (2016) [34] of “carbohydrate catabolism fueling defense mechanisms” in rice under insect stress. It is also consistent with the findings of Hartmann and Trumbore (2016) [77] on the role of non-structural carbohydrates in tree stress responses. This phenomenon exemplifies the principle of trade-offs in plant resources, where priority is given to the safeguarding of defense mechanisms and the ensuring of survival under conditions of stress.

4.4. Oxidative Stress Management: A Nexus from Passive Response to Active Defense

A particularly salient finding is the dual and significant enrichment of the “Glutathione Metabolism” pathway at both the transcriptomic and metabolomic levels. This finding indicates that borer herbivory triggers a burst of reactive oxygen species (ROS), to which sugarcane responds by rapidly activating a robust antioxidant system to maintain cellular redox homeostasis. This process not only prevents oxidative damage associated with defense reactions but may also constitute an active defense strategy in itself. For instance, glutathione and its related metabolites have been demonstrated to either directly participate in the process of detoxification or function as defensive signaling molecules. As Liu et al. (2022) [78] also emphasized in their study on quinoa, glutathione metabolism plays a pivotal role in insect resistance. Furthermore, oxidative stress is frequently associated with lipid peroxidation processes, which generate signaling molecules, such as jasmonic acid or lipid derivatives (e.g., azelaic acid), that possess direct insecticidal activity. This finding

is consistent with the observations reported by Agarrwal et al. (2016) [66] of enhanced lipid peroxidation and azelaic acid accumulation in rice during resistance to gall midges, which contributed to maggot mortality. Therefore, the management of oxidative stress, highlighted by our omics data, is not merely a passive protective response but likely a pivotal regulatory node connecting early stress perception to the execution of downstream defense programs in sugarcane under borer attack. The significant enrichment of the “Glutathione metabolism” pathway observed here aligns with findings in the sugarcane-*X. albilineans* interaction, where it was a key upregulated pathway [29], suggesting it represents a core conserved component of sugarcane’s stress response. Future research should investigate the crosstalk between this oxidative stress management system and other co-enriched signaling pathways, such as “plant hormone signal transduction” [29], to elucidate how integrated networks determine the final resistance phenotype.

5. Conclusions

In summary, our integrated transcriptomic and metabolomic analysis reveals that sugarcane mounts a robust, multi-layered defense response against borer (*C. sacchariphagus*) infestation, which exhibits significant cultivar-specificity manifesting as distinct transcriptional strategies (suppressive in Z4, activating in Z5, and balanced in Z6). This response is coordinated by a highly integrated regulatory network, initiated by hormone signaling (e.g., JA) and MAPK cascades, which drives a global transcriptional reprogramming. Consequently, primary metabolic fluxes are redirected to fuel the potent activation of biosynthetic pathways for direct defense compounds, centered on phenylpropanoids and flavonoids, while simultaneously priming mechanisms for indirect defense. Throughout this process, a delicate redox balance is maintained to ensure an effective yet controlled outcome. These findings elucidate the molecular underpinnings of induced resistance in sugarcane and provide key genetic and metabolic targets. Future research should focus on the functional validation of core regulators within this network (e.g., via gene editing) and the application of these candidates in molecular breeding programs to develop novel sugarcane varieties with enhanced and sustainable borer resistance.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy16050570/s1>, Figure S1: Chemical Structures of Representative Metabolites Highlighting Cultivar-Specific Responses to Borer Infestation.

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