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ORIGINAL ARTICLE

Transcriptome and metabolome integration in sugarcane through culm development

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Abstract

Sugarcane (Saccharum sp.) is a tropical and subtropical C4 plant with a high photosynthetic and carbon assimilation efficiency that stores sucrose. Culm biomass is also composed of bagasse fibre, a by-product of the sugarcane industry. This high-yielding grass, high in sucrose and lignocellulosic biomass, is considered an optimal feedstock as an alternative to fossil fuels and to produce a broad range of high-value biomaterials. The ideal sugarcane production system would optimise the relative production of sugar and these new products. Multi-omics correlation analysis was used to generate a global view of the essential metabolic pathways identifying critical genes involved in carbon partitioning during different stages of development. This research *employed* an unprecedented metabolic and transcriptomic dataset of 360 samples from a selection of 1440 culms of 24 genotypes at five different development stages. Chemical composition and metabolome analysis showed an increase through the culm development of lignin, sucrose, carbon, and amino acids such as aspartic acid, serine, alanine, methionine, threonine 3-cyano-L-alanine, and citric acid. Transcriptome analysis revealed functionalities such as transcription, nucleotide transport and metabolism, and the biosynthesis of amino acids that are highly activated during the immature stage and highly down-regulated during the most mature age.

KEYWORDS

bioenergy, metabolic pathways, multi-omics integration, plant, sugarcane development

1 | INTRODUCTION

Sugarcane is a semi-perennial, highly photosynthetic efficient crop with high CO2 fixation and a high yield of carbohydrates per hectare (Henry, 2010). With the ability to store high concentrations of sucrose in the cane stalks, sugarcane is a significant crop for the production of sugar, with a potential to generate large-scale recyclable bioplastic, bioenergy, and valuable by-product, as dietary supplements, sweeteners, and phytochemicals

(Hoang et al., 2015; Kiran et al., 2017; Yadav et al., 2018). Sugarcane breeding programmes are challenging, considering the genome with a size of about 10 Gb organised into around 120 chromosomes being highly polyploid, aneuploid, heterozygous (Bremer, 1961; Silva et al., 1995; Hont et al., 1996; Hont et al., 1998; Piperidis and D'Hont, 2020) and with a reference monoploid genome published only recently (Garsmeur et al., 2018; Trujillo-Montenegro et al., 2021).

A better understanding of sugarcane growth with a finer definition of links between enzymes, metabolites, and metabolic pathways through development is required to support selection in improved breeding programmes for yield, fibre quality, sugar, chemical bioproduct, and plant synthetic biology. This study reports the integration of two large datasets, metabolomics (Perlo et al., 2020), and transcriptomics (Perlo et al., 2022). These two datasets were generated from a sample collection of 1440 internodes across 24 sugarcane cultivars through five different developmental stages.

The metabolome and transcriptome are compelling datasets to identify genetic regulation to improve sugarcane productivity and specific traits (Bosch et al., 2003; Botha and Black, 2000; Carson and Botha, 2000; Jackson, 2005; Glassop et al., 2007). The multi-omics analysis highlights complementary information, with no direct association between metabolite and transcript (Cavill et al., 2016). Otherwise, integrated multi-omics high-throughput approaches with network analysis have proved efficient for crop improvement (Großkinsky et al., 2018; Shah et al., 2018; Yadav et al., 2018). Complementary information and integrated information from these different omics datasets have promising sugarcane breeding and product diversification applications. Multi-omics technologies give unprecedented opportunities to present a global view of a network of regulatory interactions, providing an apprehensive illustration to identify key biosynthesis pathways, target-specific enzymes, revealing influencing traits in polyploid plants (Civelek and Lusis, 2014; Scossa et al., 2021). With climate, economic, and social changes, there needs to be a highlight on generating predictive models from integrative multi-omics datasets to understand better the global intricate molecular mechanisms that control carbon partitioning, phenotypic changes, and response to biotic or abiotic factors stress. These models may also improve multi-trait breeding programmes, metabolic pathways, and gene expression engineering. For this research, pathway-based integration methodology was used to interpret the results, and metabolite transcripts were attempted to integrate the datasets through photosynthesis outputs produced in the growth sink of the young culm to storage in the mature stem.

2 | MATERIALS AND METHODS

2.1 | Plant materials and field experiments

The sugarcane field trial was established by Sugar Research Australia, Burdekin station, Queensland, Australia. A trial of three replicates of 24 different genotypes was planted on the 29th of August 2017. The cane stalks were collected after 19 weeks and 37 weeks of growth, in the morning, from four different stools for each plot's three replicates. The internodes 5 and 8 (third and sixth internodes below the first visible dewlap leaf) were extracted during the first and second collections. Internodes Ex5 from the second collection were the internodes 5 of the first collection, which was tagged at this period.

2.2 | Pulverisation and homogenisation

Samples were stored in a freezer at −80°C and then placed in liquid nitrogen before being inserted in the cryo-jars to be pulverised using TissueLyser (Qiagen) for 90 s at a frequency of 30 Hz. The resulting powder from the four different stools of a similar internode was homogenised and stored in −80°C freezers.

2.3 | Total lignin quantification with acetyl bromide

In a fume hood, 10 mg of biomass (lyophilised samples) was dissolved in 2 ml screw cap tubes with a 1 ml of 25% AcBr / 75% glacial acetic solution. The samples were incubated for 2 hours at 50°C with constant stirring in a rotating hybridisation oven and then placed in a 4°C fridge for about 5 min. The samples were centrifuged at 20,000*g* for 5 min. The solution was transferred into a 96-well UV plate in duplicates. For each plate, duplicate standards with 1, 2, 4, and 6 µl of 10 mg lignin solution have received the same treatment as samples. In each well, 60 µl of the solution named acetic acid NaOH hydroxylamine Mastermix (48 µl acetic acid, 9.5 µl 2 M NaOH and 1.7 µl 0.5 M hydroxylamine) was added. Finally, 200 µl glacial acetic was also added to each well. The UV spectrum was measured at 280 nm.

2.4 | Protein

The elemental (CHN) analysis directly on the biomass was performed on a Thermo Scientific Flash Smart CHNS analyser at the University of California, Berkeley, following

the National Renewable Energy Laboratory procedures. Protein content was estimated by a nitrogen-to-protein conversion factor of 6.25.

2.5 | Metabolome

GCMS profiling was performed by Metabolomics, University of Melbourne, Australia, as described in Marquardt et al. (2019). Detailed preparation of samples for extraction of metabolites was described in Perlo et al. (2020).

2.6 | Transcriptome

Total RNA was extracted from approximately 2.5 g of frozen powder for each internode using TRIzol reagent (Invitrogen). The RNA supernatant was treated with Qiagen RNeasy Plant Mini Kit (Qiagen) for RNA purification (Furtado, 2013). The RNA concentration was measured by spectroscopy with NanoDrop. A260/280 ratios were approximately 2.1 and not below 1.8. The RNA integrity number (RIN) checked with an Agilent Bioanalyzer for each sample was higher than 0.8 and stored in a −80°C freezer. Three μg of RNA was used for library preparation and sequencing, processed for the 360 samples by SCI Ramaciotti Centre for Genomics, UNSW, Sydney, Australia, on NovaSeq™ 6000 Illumina Sequencing System.

2.7 | Differential expression analysis

Using CLC Genomics Workbench 12.0.3, adaptors and low-quality bases were trimmed from raw reads with a cut-off of 0.01, and quality control checks on raw sequence data were assessed. The FASTQ sequence reads were aligned to sugarcane transcriptome references generated with PacBio RSII (Hoang et al., 2017) to create a table of counts with the Create Expression Browser tool of CLC. A matrix of total counts showing the expression levels of mRNA, TPM (transcripts per kilobase million) of 107,598 genes was generated for our 360 samples.

The count table was loaded in OmicsBox 1.1.164, where the Differential Gene Expression (DGE) analysis was generated with FDR (false discovery rate) p-value ≤ 0.05 and log fold change abs value >1, TMM (Trimmed mean of M values) normalisation method and GLM (likelihood ratio test) statistical test. DGE was realised using systematically oldest versus youngest stages. Functional annotation based on orthology assignments was performed with Eggnog-mapper v1 (Huerta-Cepas et al., 2017). The Gene Ontology annotation and KEGG pathway enrichment analysis of genes were performed by KOBAS version 3.0 (http://kobas.cbi.pku.edu.cn/). Data synthesis was displayed using the pheatmap package (Kolde, 2012). KEGG pathways were shown with the KEGG Mapper platform version 5 (http:/www.kegg.jp/kegg/tool/map_pathway.html). Pearson's correlation network was realised with SparCC (Friedman and Alm, 2012). Inter-omics correlation network analysis was realised using weighted gene co-expression network analysis, R package version 1.69 (Zhang et Horvath, 2005; Langfelder et Horvath, 2008). The 100 highest positive and negative correlations between genes and the metabolites were integrated into Cytoscape 3.9.1 (Shannon et al., 2003).

3 | RESULTS

The composition of the sugarcane culm, including lignin, protein, and metabolite concentrations, was compared at the different development stages. The total lignin content of the stems was measured at these different development stages. The analysis revealed that the lignin content was significantly higher in the lower mature internodes than in the immature top internodes. These results were consistent for the two collections (Figure 1a). For the same internode (internode 5 or internode 8) at different periods (collection 1 compared to collection 2), the percentages of lignin were not significantly different.

The carbon concentration was higher in the mature internodes than in the more immature internodes. This same trend was also measured for hydrogen with no significant difference. In contrast, the protein concentration decreased with maturity. More mature internodes had the lowest concentrations of proteins. Other compounds referred to as non-protein nitrogen, such as free amino acids, nucleotides, creatine, and choline, decreased during the plant's maturation (Figure 1b).

The metabolite analysis, using heatmap and dendrogram of the hierarchical cluster analysis (Figure 2a) of the relative concentration of 73 metabolites, showed a clear grouping of the samples per internodes maturation. The immature samples (internodes 5) from the two collections (19 weeks and 37 weeks) were clustered together, and the more mature samples (internodes 8) from the 2 collections were clustered together. The oldest and most matured (internodes Ex5) samples were clustered separately. This heatmap also revealed two essential metabolites based on their profiles (hierarchy on the left of the heatmap). The first one, on the top, exposed a group of metabolites with the highest concentrations in immature internodes (internodes 5 from the first and second collections). In contrast, the second cluster showed an opposite profile, with most

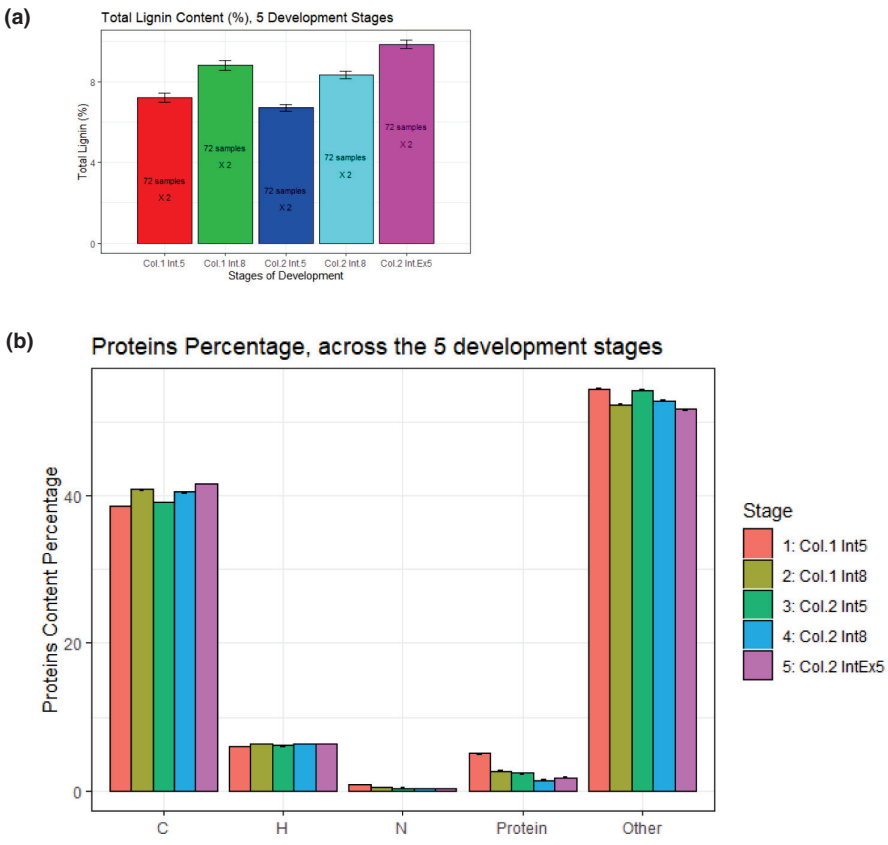

FIGURE 1 (a) Lignin content at different stages of development. Error bars show standard deviation ($n = 144$). Two technical replicates of the 72 samples (24 genotypes, 3 biological replicates). (b) Carbon (C), hydrogen (H), nitrogen (N), proteins and other content (percentage), during 5 different development stages, using analytical methods. Error bars show standard deviation ($n = 144$).

metabolites with the highest concentrations in the lower, most mature internodes (Internode Ex5).

Pearson's correlation network revealed three essential clusters. The first group included caffeic acid and itaconic acid. The second regrouped tyrosine, myo-inositol, hexadecanoic acid, and 3-coumaric acid. The third and largest group, showing a relationship with the two other groups, was represented by alanine, 3-cyano-L-alanine, asparagine, ribose, threonine, isoleucine, 4-amino butanoic acid, malic acid, threonic acid, glucuronic acid, and galactinol (Figure 2b).

Differences in gene expression analysis revealed changes of up- and down-regulated genes through different development stages. DEG profiles were more similar between closer development stages, such as internodes 8 versus internodes 5 from the first collection (19 weeks old) and internodes 8 versus internodes 5 from the second collection (37 weeks old), than the most mature internodes Ex5 versus internodes 8 of the second collection. Also, interestingly 696 up- and 825 down-regulated genes were conserved during all development stages. The highest number of DEGs was specific to earlier stages of development (Figure 3). This analysis completes the results of the sugarcane transcriptome by Perlo et al. (2020). Differentially expressed gene numbers were highest during the first stage of maturity and age. Comparing internodes 8 versus internodes 5 after 19 weeks of growth (2vs1) revealed 16,393 DE genes, with 55.4% down-regulated and 44.6% up-regulated genes. After 37 weeks, DEG between internodes 8 versus internodes 5 (4vs3) showed a lower number; 12,240 DE genes with 55% down-regulated and finally comparison of the oldest and most mature internode Ex5 compared to internode 8 (5vs4) exposed only 4707 differentially expressed genes with 60% of genes down-regulated.

Clusters of Orthologous Genes (COG) annotation analysis revealed that functional and storage activities diverged considerably during the development stages of the plant. Functions such as translation, ribosomal structure and biogenesis, RNA processing and modification, transcription, replication, recombination and repair, nuclear structure, energy production and conversion were up-regulated during the development of the sugarcane. Oppositely, the function of cytoskeleton, extracellular structures, carbohydrate transport and metabolism, amino acid transport and metabolism, lipid transport and metabolism, inorganic ion transport, and metabolism decreased during the development stages. Interestingly, the function of transcription, replication, recombination, and repair, signal transduction mechanisms and nucleotide transport and metabolism were highly up-regulated during the young age of the sugarcane, when the function of translation, ribosomal structure, and biogenesis was most expressed during the oldest age (Figure 4).

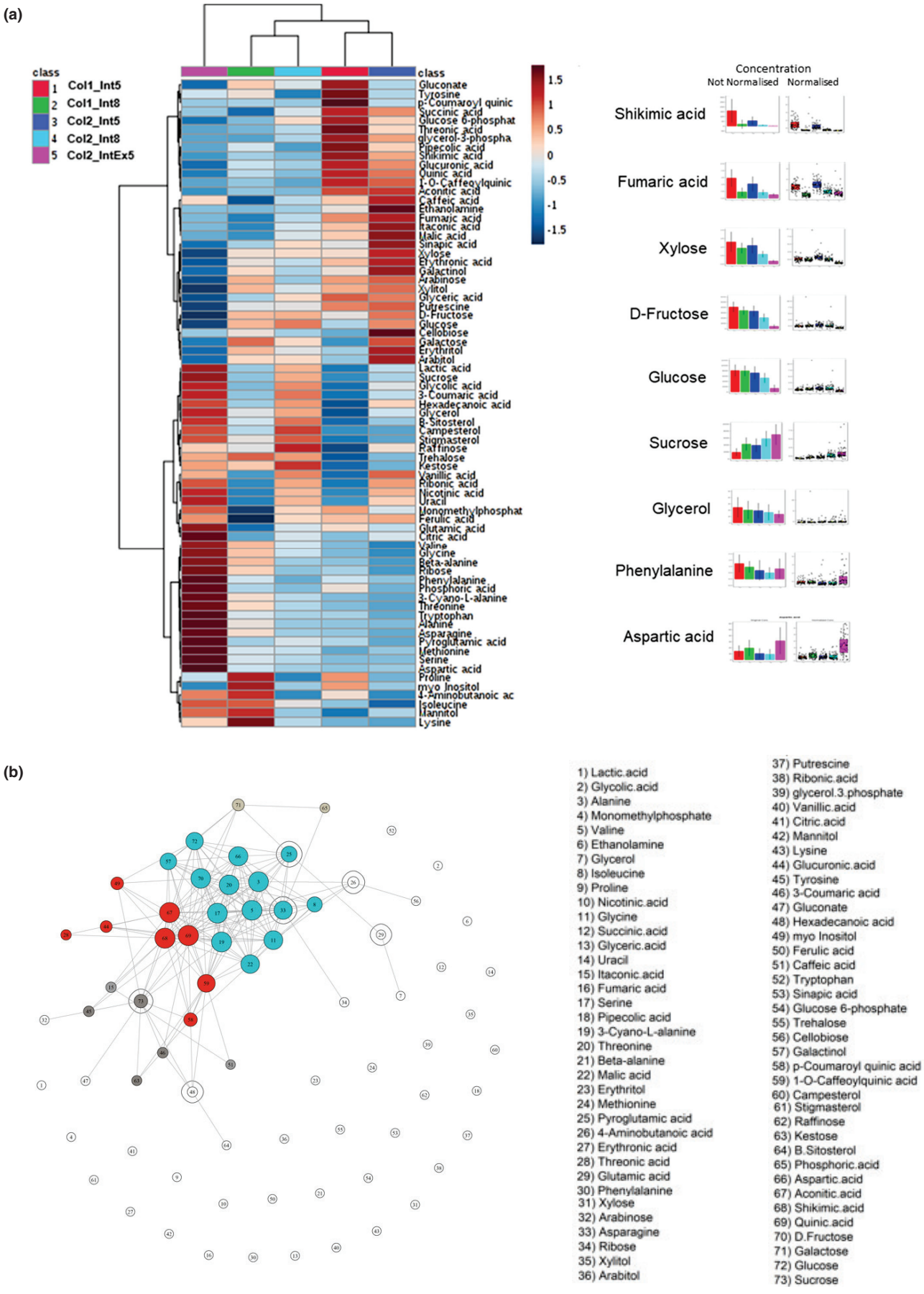

FIGURE 2 (a) Hierarchical cluster analysis (Euclidean distance ward. D clustering algorithm). Color scale displays relative normalised concentrations of metabolites for each stage. Relative original and normalised concentrations of 9 metabolites have been highlighted (on the right). (b) Correlation network of the relative normalised concentration of 73 metabolites, using Pearson's correlation coefficients.

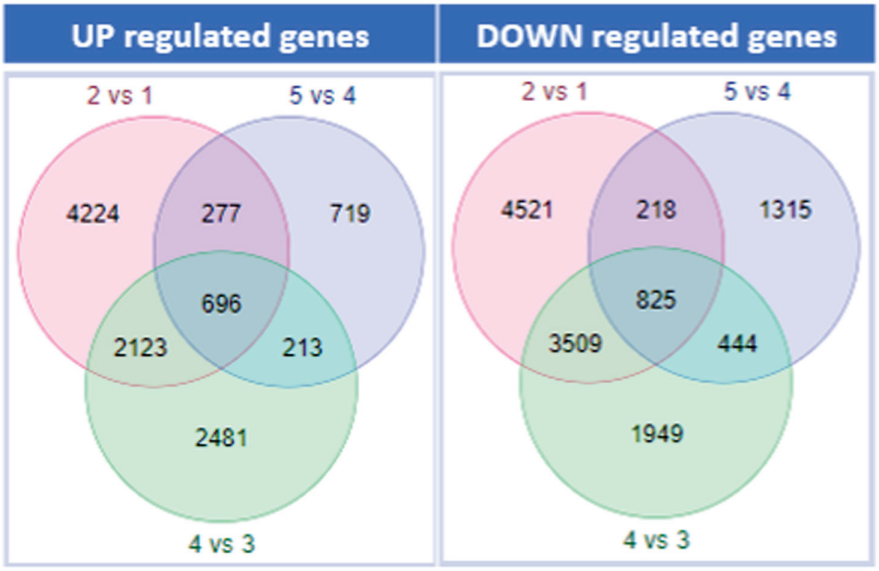

FIGURE 3 Venn diagram summarising up and down genes during the different development stages. Stages from 1 to 5 increase from youngest and most immature to older to more mature. 2vs1: Internode 8 versus internode 5, 19 weeks old; 4vs3: Internode 8 versus internode 5, 37 weeks old and 5vs4: Internode Ex5 versus internode 8, 37 weeks old Stage 1: Internode 5 from collection 1 (19 weeks old), stage 2: Internode 8, collection 1, stage 3: Internode 5 from collection 2 (37 weeks old), stage 4: Internode 8, collection 2, stage 5: Internode Ex5, collection 2.

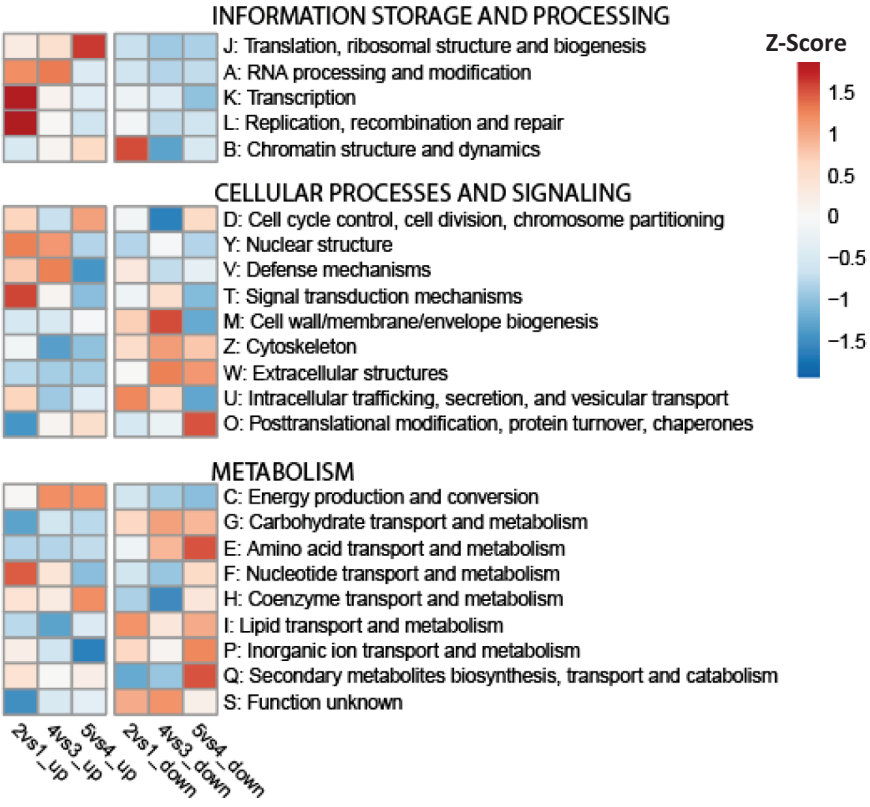

FIGURE 4 Heatmap representation of Clusters of Orthologous Groups (COG) functional classification of the up and down differentially expressed genes (DEG) at different development stages. Stage 1: Internode 5 from collection 1 (19 weeks old), stage 2: Internode 8–19 weeks old, stage 3: Internode 5 from collection 2 (37 weeks old), stage 4: Internode 8–37 weeks old), stage 5: Internode Ex5–37 weeks old.

KEGG Pathway enrichment analysis revealed apparent diversity in the biological function enrichment across development stages (Figure 5). The pathways with the proportion of most up-regulated genes during the youngest age, 19 weeks old, were Biosynthesis of amino acids, cyanoamino acid metabolism, cysteine and methionine metabolism, phenylpropanoid biosynthesis, phenylalanine, tyrosine and tryptophan biosynthesis, and also circadian rhythm – plant, glycolysis/gluconeogenesis, starch and sucrose metabolism, fructose, and mannose

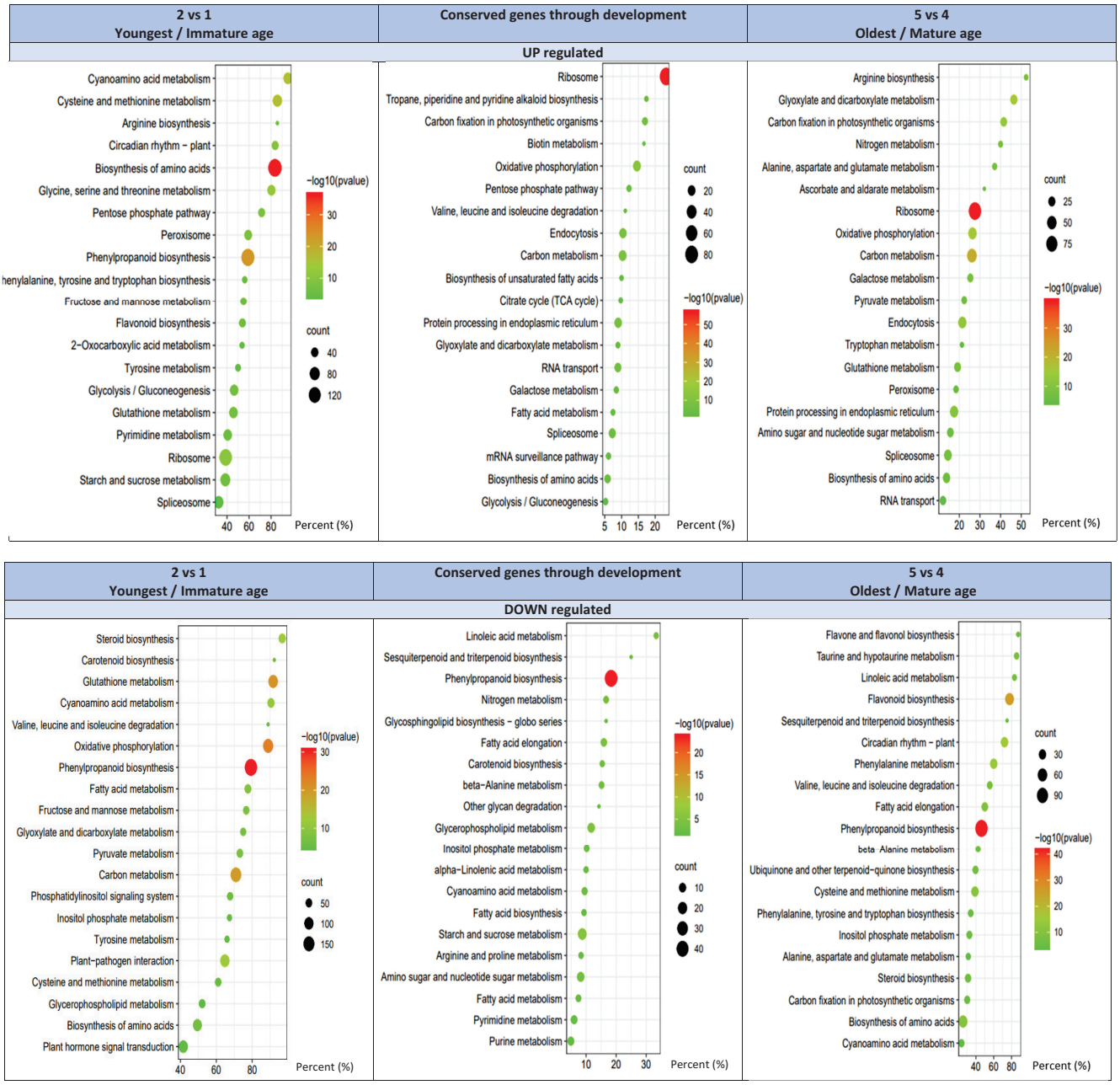

FIGURE 5 KEGG, functional enrichment pathways. Bubble diagrams representation of up- and down-regulated genes in metabolic pathways through different development stages. Lists the top 20 KEGG pathways ranked with the smallest *p*-value (or Q value) represented for DEG at youngest/immature development age, week 19_Int 8 vs week 19_Int 5 (2 vs 1), genes conserved during all development (4vs3_VS_2vs1__VS__5vs4), DEG at oldest age/mature development, week 37_Int Ex5 VS week 37_Int 8 (5 s 4).

metabolism. Up-regulated genes were more assigned to nitrogen metabolism at the oldest and most mature age. Up-regulated genes conserved during all of the development were genes involved in ribosome, carbon fixation in photosynthetic organisms, carbon metabolism, citrate cycle, pentose phosphate pathway, biosynthesis of unsaturated fatty acids and biotin metabolism. Down-regulated genes were more represented in steroid biosynthesis at the youngest age, 19 weeks old. The activity of the phenylpropanoid biosynthesis decreased during the development. Flavonoid, flavone, and flavonol biosynthesis were highly down-regulated at the mature stage (Figure 5).

Metabolome and transcriptome association of the central pathways during the development of the sugarcane culm were analysed. Illustration of the starch and sucrose metabolism revealed a significant increase in sucrose concentration during the development of the plant. The trehalose concentration was stable during the first stage of the plant increased during the oldest and most mature stage, while D-glucose decreased during

the oldest age. Key enzymes, such as UDP-glucose pyrophosphorylase (UGP2), were up-regulated during all the stages of maturity. Interestingly, sucrose-phosphate phosphatase (SPP), alpha-glucan branching enzyme (glgB), alpha-amylase (AMY), beta-amylase or BMY, glycogen phosphorylase enzymes were highly up-regulated during the oldest stage of the plant. The concentration of glucose decreased at the oldest stage of the plant. Following the same trend, the enzyme endoglucanase was down-regulated during the development of the sugarcane culm, as were maltase-glucoamylase, fructokinase or hexokinase, and EGLC (glucan endo-1,3-beta-D-glucosidase) (Figure 6).

Analysis of the phenylpropanoid biosynthesis pathway revealed many enzymes with a decrease in expression at the oldest age. Some of them were highly expressed at the youngest stage, such as phenylalanine/tyrosine ammonia-lyase (PTAL), caffeoyl-CoA O-methyltransferase (CCoAOMT), and ferulate-5-hydroxylase (Figure 7).

Analysis of the phenylalanine, tyrosine, and tryptophan biosynthesis revealed that tryptophan and phenylalanine increased during the plant's maturation. Similarly, the aspartate aminotransferase was up-regulated during maturation (Figure 8).

Analysis of carbon fixation in photosynthetic organisms displayed enzymes such as RUBISCO which were highly up-regulated at the young and intermediate age of the plant to be down-regulated at the oldest and mature age. Fructose −1,6-bisphosphatase (F2KP) and alanine transaminase were up-regulated during the development of the plant. Sedoheptulose-bisphosphatase, malate dehydrogenase, pyruvate, phosphate dikinase, and aspartate aminotransferase were all up-regulated at the oldest stage. The carbon fixation in photosynthetic organisms with the

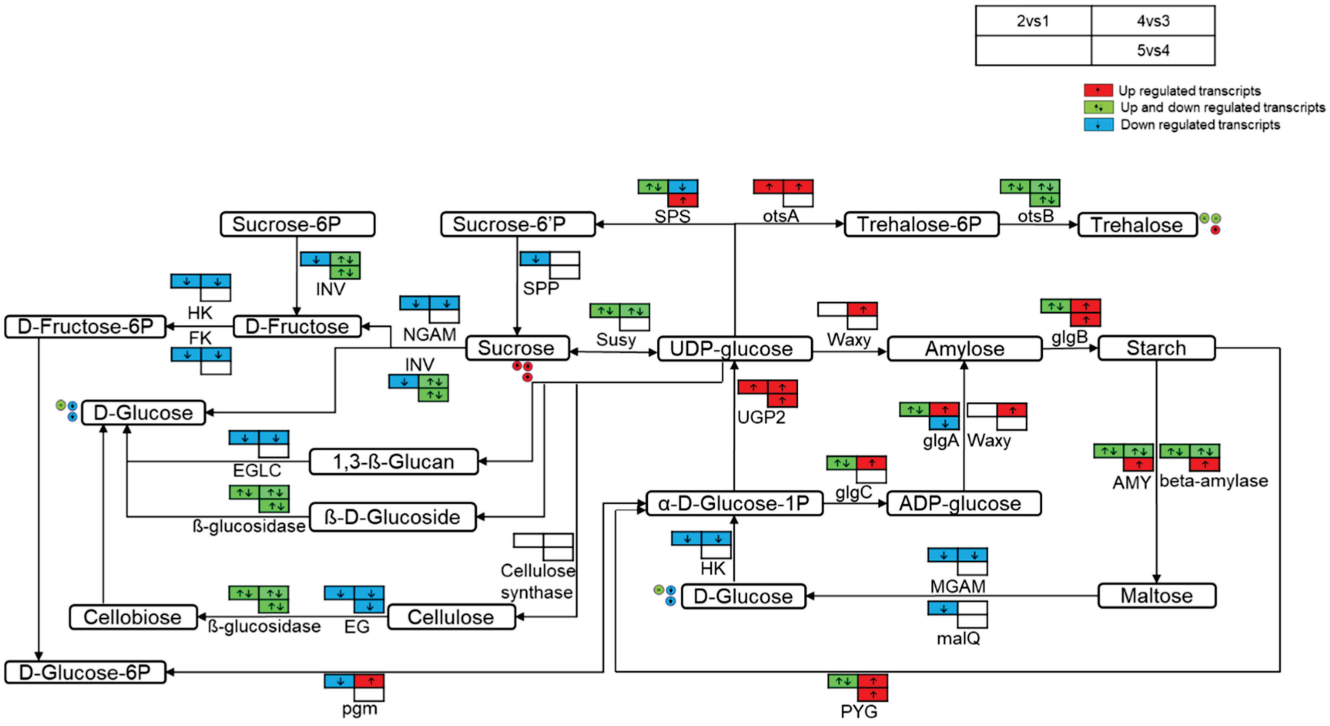

FIGURE 6 Schematic view of the starch and sucrose metabolism with different genes expression (square boxes) and different metabolite expressions (red: Significantly up-regulated, green or blue circle: Significantly down-regulated) between three different development stages. 2vs1: Internode 8 versus internode 5, 19 weeks old; 4vs3: Internode 8 versus internode 5, 37 weeks old and 5vs4: Internode Ex5 versus internode 8, 37 weeks old Stage 1: Internode 5 from collection 1 (19 weeks old), stage 2: Internode 8, collection 1, stage 3: Internode 5 from collection 2 (37 weeks old), stage 4: Internode 8, collection 2, stage 5: Internode Ex5, collection 2. Red squares and circles represent up-regulation, blue down-regulation. Green squares for the gene expression represent a mix of gene up- and down-regulated; green circles represent no variation of metabolite concentration. Susy: sucrose synthase [EC:2.4.1.13], HK: hexokinase [EC:2.7.1.1], FK: fructokinase [EC:2.7.1.4], pgm: phosphoglucomutase [EC:5.4.2.2], Waxy: granule-bound starch synthase [EC:2.4.1.242], glgC: glucose-1-phosphate adenylyltransferase or ADP glucose pyrophosphorylase [EC:2.7.7.27], glgA: starch synthase [EC:2.4.1.21], glgB or GBE1: 1,4-alpha-glucan branching enzyme [EC:2.4.1.18], ISA: isoamylase [EC:3.2.1.68], AMY: alpha-amylase [EC:3.2.1.1], MGAM: maltase-glucoamylase [EC:3.2.1.20 3.2.1.3], PYG or glgP: glycogen phosphorylase [EC:2.4.1.1], SPS: sucrose-phosphate synthase [EC:2.4.1.14], EGLC: glucan endo-1,3-beta-D-glucosidase [EC:3.2.1.39], EG: endoglucanase [EC:3.2.1.4], otsA: trehalose 6-phosphate synthase [EC:2.4.1.15 2.4.1.347], otsB: trehalose 6-phosphate phosphatase [EC:3.1.3.12], SPP: sucrose-phosphate phosphatase [EC:3.1.3.24], bcsA: cellulose synthase (UDP-forming) [EC:2.4.1.12].

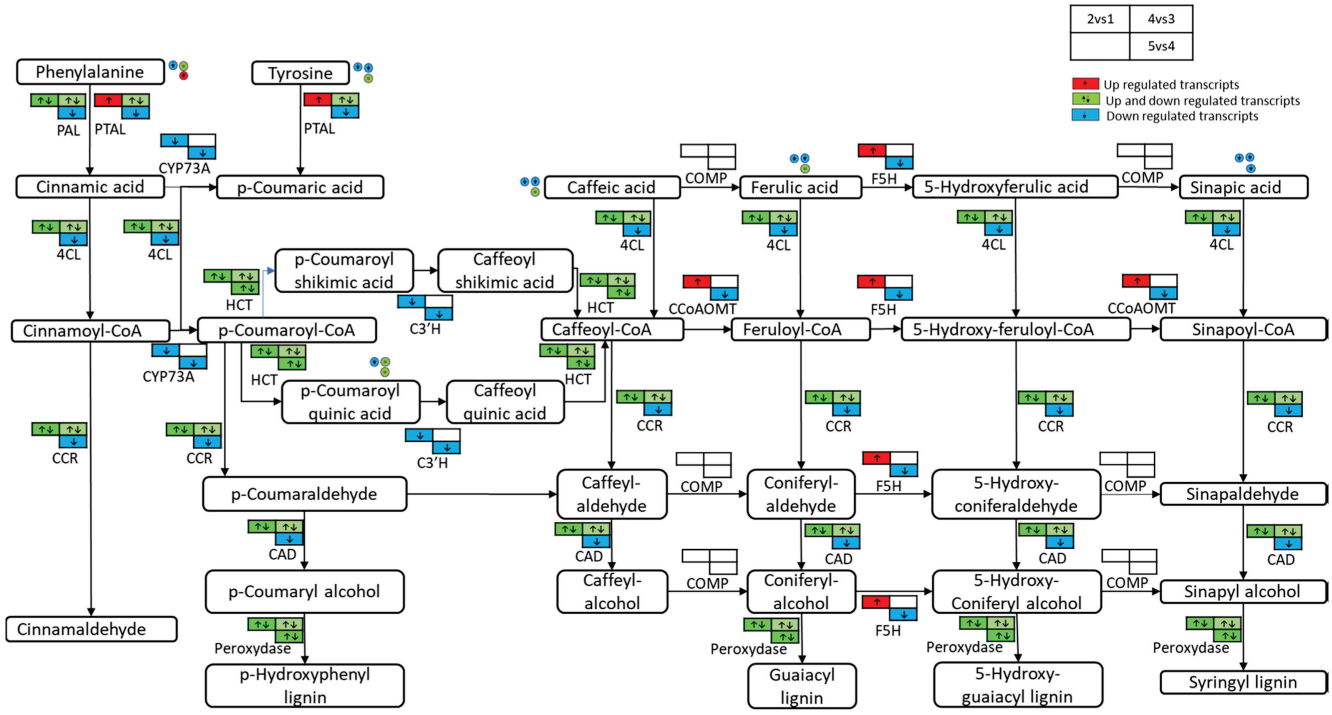

FIGURE 7 Schematic view of the phenylpropanoid biosynthesis pathway with significant differences in gene expression (square boxes) and significant differences in metabolite expression (red, green, or blue circle) between three different development stages. 2vs1: Internode 8 versus internode 5, 19 weeks old; 4vs3: Internode 8 versus internode 5, 37 weeks old and 5vs4: Internode Ex5 versus internode 8, 37 weeks old Stage 1: Internode 5 from collection 1 (19 weeks old), stage 2: Internode 8, collection 1, stage 3: Internode 5 from collection 2 (37 weeks old), stage 4: Internode 8, collection 2, stage 5: Internode Ex5, collection 2. Red squares and circles represent up-regulation, blue down-regulation. Green squares for the gene expression represent a mix of gene up- and down-regulated, and green circles represent no variation of metabolite concentration. F5H: ferulate-5-hydroxylase, 4CL: 4-coumarate-CoA ligase [EC:6.2.1.12], HCT: shikimate O-hydroxycinnamoyltransferase [EC:2.3.1.133], CYP73A: trans-cinnamate 4-monooxygenase [EC:1.14.14.91], PAL: phenylalanine ammonia-lyase [EC:4.3.1.24], PTAL: phenylalanine/tyrosine ammonia-lyase [EC:4.3.1.25], CCR: cinnamoyl-CoA reductase [EC:1.2.1.44], CCoAOMT: caffeoyl-CoA O-methyltransferase [EC:2.1.1.104], CAD: cinnamyl-alcohol dehydrogenase [EC:1.1.1.195].

C4-dicarboxylic acid cycle was constantly active to produce amino acids and C compounds (Figure 9).

Inter-omics correlation network analysis between transcriptome and metabolome showed a strong positive correlation between critical genes and metabolites. Some metabolites such as fructose and tyrosine were associated with a low number of genes, respectively, KCS1 and CCR1 and ALPHA-VPE, when essential genes such as KCS11 and MYB12 XYL4 were connected to many metabolites. Genes negatively correlated to metabolites described another profile with multiple genes linked to fructose, such as TEF1, TOC1, GA20ox1, THI1, cox2, HRR23, and psaA. Genes with the highest rank of negative correlation with metabolites were more specific, and fewer genes were negatively linked to these different metabolites (Figure 10).

4 | DISCUSSION

The development of sugarcane depends on carbon (C) and nitrogen (N) assimilation. This study showed that the carbon concentration increased with age and maturity while the protein/nitrogen ratio decreased during this period. These results reflected the presence of nitrogen in the amino acids and nucleotides, which were essential for synthesising proteins and nucleic acids (Nunes-Nesi et al., 2010; Paungfoo-Lonhienne et al., 2010). With the promise of the transformation of bagasse as lignocellulosic biomass to produce biofuels and biochemical products, the lignin biosynthesis pathway has been more widely studied in sugarcane (Bottcher et al., 2013; Kasirajan et al., 2018; Lingle & Thomson, 2012; Mason et al., 2020; Vicentini et al., 2015) together with the precursor shikimate pathway (Vogt, 2010). This study confirmed a constant increase in lignin concentration during the development of the plant. These results were consistent with the youngest upper internodes being primary growth sinks, while the mature bottom internodes are the stems where carbohydrate reserves are stored (Botha et al., 2000; Moore, 1995; Wang et al., 2013). At the same time, we saw with the metabolome analysis that a few compounds, sucrose and essential amino acids, such as

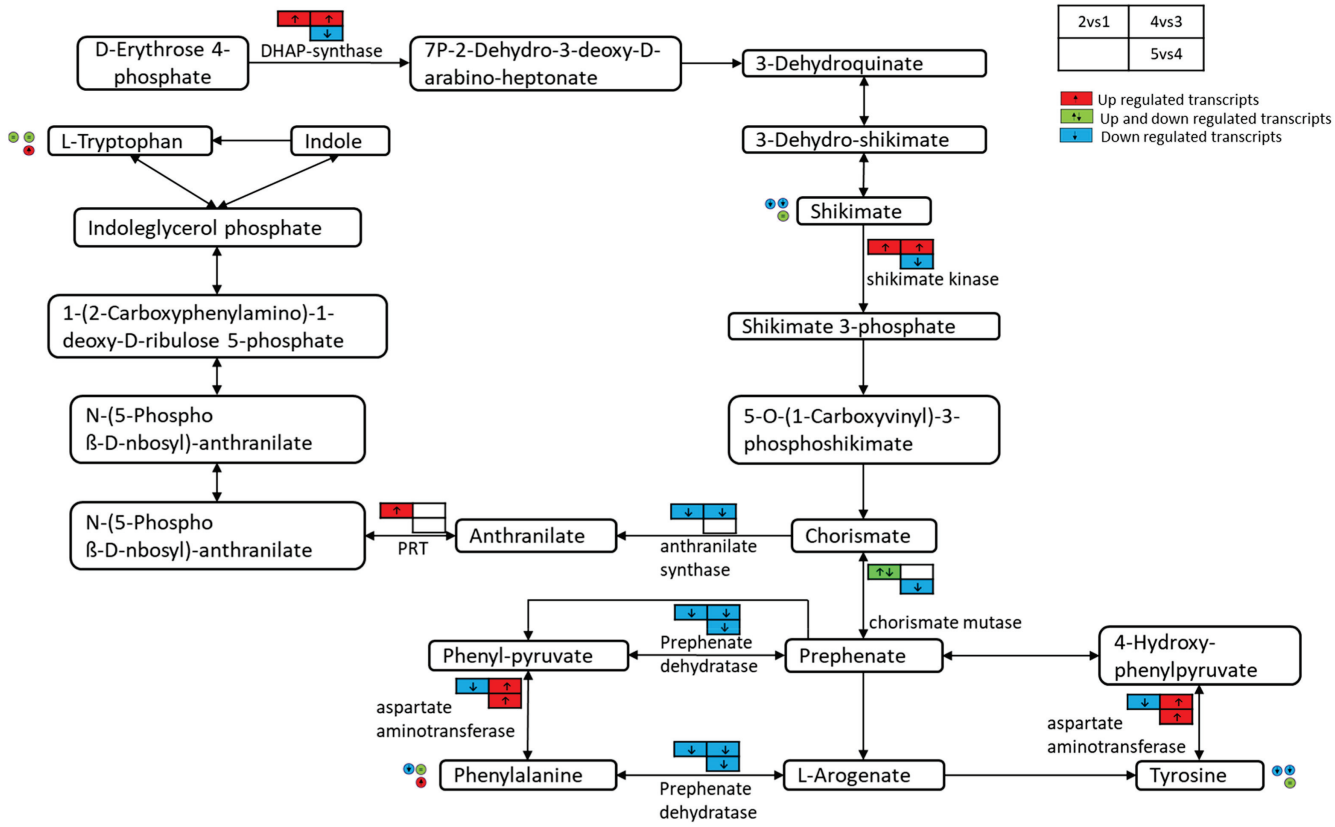

FIGURE 8 Schematic view of the phenylalanine, tyrosine, and tryptophan biosynthesis with significant differences in gene expressions (square boxes) and significant differences in metabolite expressions (red, green or blue circle) between three different development stages. 2vs1: Internode 8 versus internode 5, 19 weeks old; 4vs3: Internode 8 versus internode 5, 37 weeks old and 5vs4: Internode Ex5 versus internode 8, 37 weeks old. Stage 1: Internode 5 from collection 1 (19 weeks old), stage 2: Internode 8, collection 1, stage 3: Internode 5 from collection 2 (37 weeks old), stage 4: Internode 8, collection 2, stage 5: Internode Ex5, collection 2. Red squares and circles represent up-regulation, blue down-regulation. Green squares for the gene expression represent a mix of gene up- and down-regulated, and green circles represent no variation of metabolite concentration. PRT: anthranilate phosphoribosyltransferase [EC:2.4.2.18].

aspartic acid, serine, alanine, and methionine, increased during the development of the plant. Amino acids such as alanine and methionine have contributed to plant growth (Alfosea-Simón et al., 2020; Khan et al., 2000). Different delays of amino acid degradation can also explain these results (Hildebrandt et al., 2015). With different patterns, all the other compounds analysed showed decreased concentration. This decrease was also correlated with a clear trend of decreasing gene expression during the development of the sugarcane culm. COG functional activities such as transcription, replication, DNA recombination, and repair were highly represented during the youngest stage, ensuring fundamental genetic process and maintenance of the genome integrity (Costa et al., 2001). During the youngest stage, the highest general metabolic activity indicated that DNA recombination and repair activity was highest at this stage. It has been suggested that homologous recombination repair required to maintain genomic activity decreases with age with a general reduction of the DNA repair pathways (Golubov et al., 2010; Manova and Gruszka, 2015). We may also hypothesise that this may be due to the sugarcane being grown in suitable conditions with minimal biotic and abiotic stress during this experiment, causing minimum DNA damage supporting a low transcription, replication, DNA recombination, and repair activity (Menossi et al., 2008; Costa et al., 2001). Translation, ribosomal structure, and biogenesis functionalities were age-dependent and highly expressed during the sugarcane culm's oldest stage, which may be explained by the ability of the sugarcane to maintain proteostasis to face ageing post-transcriptional controls.

KEGG enrichment pathways analysis in the phenylpropanoid biosynthesis revealed the most down-regulated genes during the oldest age. Lignin production derived from this pathway was highly activated at the youngest age and regularly decreased when lignin was stored. Similarly, the flavonoid biosynthesis was up-regulated during the youngest age to be down-regulated during the oldest age. These results are valuable in manipulating the phenylpropanoid and flavonoid biosynthesis to increase

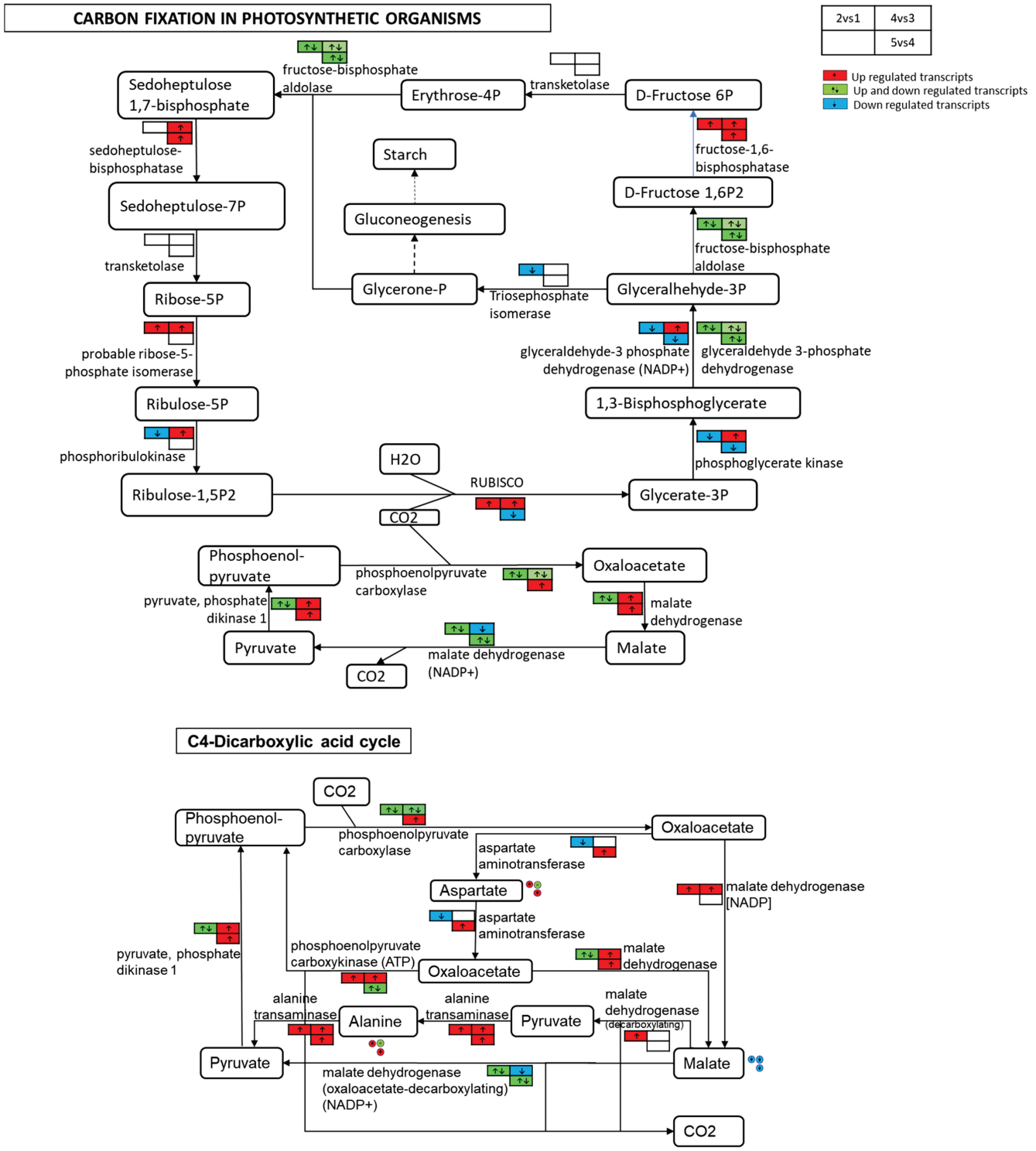

FIGURE 9 Schematic view of carbon fixation in photosynthetic organisms with C4-dicarboxylic acid cycle with significative difference in genes expression (square boxes) and in metabolite expression (red, green, or blue circle) between three different development stages. 2vs1: Internode 8 versus internode 5, 19 weeks old; 4vs3: Internode 8 versus internode 5, 37 week old and 5vs4: Internode Ex5 versus internode 8, 37 week old, Stage 1: Internode 5 from collection 1 (19 weeks old), stage 2: Internode 8, collection 1, stage 3: Internode 5 from collection 2 (37 weeks old), stage 4: Internode 8, collection 2, stage 5: Internode Ex5, collection 2. Red squares and circles represent up-regulation, blue down-regulation. Green squares for the gene expression represent a mix of gene up- and down-regulated, green circles represent no variation of metabolite concentration. RUBISCO: ribulose 1,5-bisphosphate carboxylase/oxygenase [EC 4.1.1.39].

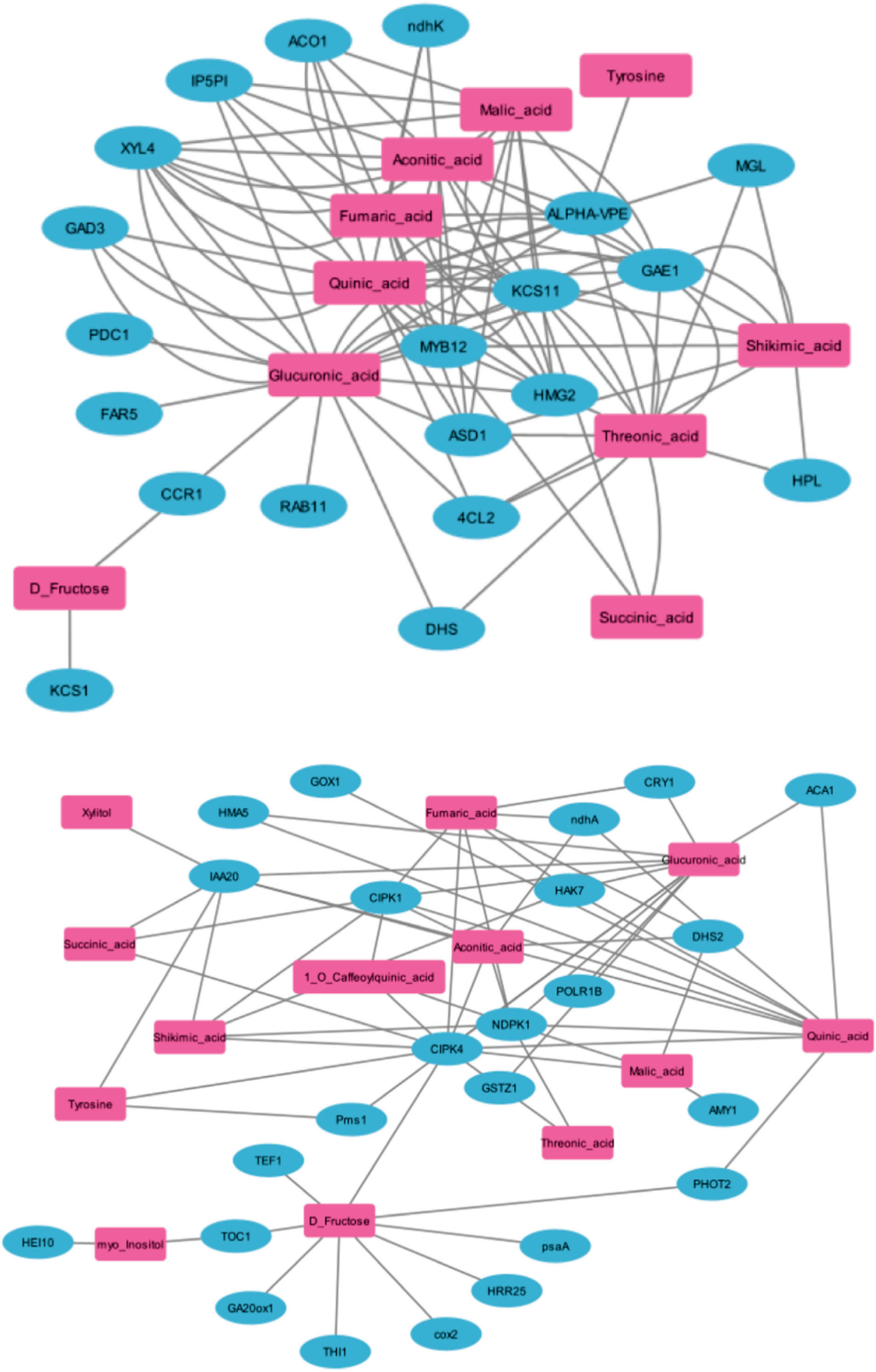

FIGURE 10 Inter-omics correlation network. Genes with the highest significative positive correlation (gene significance) to metabolites. On the top highest positive correlation, on the bottom negative correlation.

or target the generation of specific high-value metabolites such as colourants, fragrances, and biofuels (Ferreira and Antunes, 2021; Lyu et al., 2022).

The biosynthesis of amino acid, glycolysis/gluconeogenesis, starch, and sucrose metabolism were more expressed during the youngest age. The essential function to produce sucrose was highly defined at the start of development to be more constant with the storage of the metabolites generated by these pathways. Interestingly, genes involved in ribosomes were highly expressed during all the development stages of the sugarcane culm. This result revealed that mRNA translation and protein production might be high throughout development.

Phenylpropanoid biosynthesis at these different stages revealed essential enzymes, such as DAHP synthase, shikimate kinase, F5H (ferulate-5-hydroxylase), PTAL (phenylalanine/tyrosine ammonia-lyase), CCoAOMT, RUBISCO, T6P (Trehalose 6-phosphate) were highly up-regulated during the young age and highly down-regulated during the old mature age. In contrast, in the starch and sucrose metabolism, SPS (sucrose-phosphate synthase) and aspartate aminotransferase were down-regulated at the youngest age to be up-regulated at the oldest, mature age. These results confirmed the complexity of SPS activities (Heldt and Piechulla, 2021; Sharkey et al., 2000) and confirmed SPS implication in sucrose accumulation and sucrose storage in the sugarcane culm (Zhu et al., 1997). The metabolome also showed an increasing quantity of phenylalanine, while the amount of sucrose was constantly increasing throughout development. Sucrose and related enzymes such as UDP-glucose pyrophosphorylase (UGPase), which produces UDP-glucose, a precursor of sucrose and polysaccharide synthesis, were up-regulated during maturation. UGPases are critical precursors for sucrose metabolism and cell wall biosynthesis. In plant leaves, UGPase is involved in the sucrose biosynthesis pathway, providing UDPG for SPS (Kleczkowski et al., 2004). At the most mature age, key enzymes involved in the synthesis and breakdown of sucrose in sugarcane and glgB involved in glycogen biosynthesis were most vigorous. The increase of SPS at a mature age has been identified in different sorghum genotypes (Li et al., 2019). These results suggest that sucrose synthesis was still highly active during old age. In grape berry development, a positive correlation between sucrose and AMY and BMY may support these observations (Zhu et al., 2017). Invertase enzyme, an enzyme that cleaves the sucrose into hexoses as a source of energy, respiration, and carbon source for the biosynthesis of other compounds (Ansari et al., 2013; Stein and Granot, 2018), was down-regulated at the youngest age and stayed constant during late development suggesting a low utilisation of sucrose at the youngest age, increasing storage. In the phenylpropanoid biosynthesis pathway, the enzyme PTAL involved in reducing phenylalanine and tyrosine was highly up-regulated at the youngest age of the plant. Consistently, these two metabolites, phenylalanine and tyrosine decreased during this period. The results suggest the transformation of the metabolites by these enzymes. Similarly, caffeic acid and ferulic acid decreased at the youngest age when F5F was the most active, indicating a higher activity of the first steps of the phenylpropanoid biosynthesis at this youngest age. Phenylalanine, tyrosine, and tryptophan biosynthesis or the shikimate pathway is essential as a precursor of the phenylpropanoid pathways and a precursor of high-value commercial and critical defence and development plant compounds (Herrmann and Weaver, 1999). Up-regulation of the aspartate aminotransferase enzyme that transforms phenylpyruvate to phenylalanine with the consistent increase of phenylalanine and tyrosine concentrations may be relevant to producing these valuable amino acids. Aspartate aminotransferase plays an essential role in regulating C and N metabolism and biosynthesis of aspartate and amino acid biosynthesis pathways (de la Torre et al., 2014).

Carbon fixation is the conversion of inorganic carbon (CO_2) to organic compounds. PEP carboxylase has an essential role in ensuring the conversion of CO_2 to oxaloacetate, which is reduced to malate using NADP-malic enzyme and aspartate by transamination with aspartate aminotransferase. The role of RUBISCO for CO_2 assimilation (ribulose-1,5-bisphosphate carboxylase/oxygenase) is also essential (Bar-Even et al., 2011; Leegood, 2002; Ludwig, 2016). In this study, the carbon pathways were represented by strongly up-regulated enzymes able to convert CO_2 to pyruvate during the entire development of the sugarcane.

Integration between the transcriptome and metabolome using weighted gene co-expression network analysis revealed new associations between genes and transcripts. For example, fructose was positively correlated to cinnamoyl CoA reductase (CCR1) and 3-ketoacyl-CoA synthase (KCS1). CCR1 is involved in phenylpropanoid biosynthesis and has been described to be required for lignin biosynthesis in Arabidopsis (Xue et al., 2015). KCS1 is involved in fatty acid elongation. Generation of mutant with CCR1 and KCS1 knockdown may show interconnections between the different pathways that may confirm and explain these results. This analysis using high throughput omics analysis may give opportunities in synthetic biology and metabolic engineering (Mortimer, 2019).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of competing interests.

DATA AVAILABILITY STATEMENT

All data is publicly available either in the manuscript or linked to earlier metabolomics and transcriptomics papers on this topic.

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