Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Integrated Transcriptomic and Metabolomic Analysis Reveals the Amino Acid Biosynthesis Diversity in Black and White Mulberry

Zhichao Sun
Chengde Medical University
Weizhen Zhang
Chengde Medical University
Ruotong Zhang
Chengde Medical University
Saravana Kumar R.M.
Saveetha Institute of Medical and Technical Sciences
Jisheng Li
jshlee@163.com
Chengde Medical University

Research Article

Keywords: metabolome, transcriptome, amino acid biosynthesis, mulberry

Posted Date: May 15th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9333583/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at BMC Plant Biology on August 27th, 2026. See the published version at https://doi.org/10.1186/s12870-026-09692-3.

Abstract

Mulberry fruits are recognized as highly nutritious dietary foods, offering a wide range of essential nutrients, antioxidants, and bioactive compounds that contribute to overall health. The nutritional value of the mulberry fruit is mainly attributed by the type and content of amino acids which largely affects the purchasing behavior of consumers. Widely targeted metabolomic and transcriptomic analysis were used to evaluate the quality characteristics of black and white mulberry amino acids. The results showed 65 different kinds of amino acids and related metabolites accumulate at mulberry developmental stages. The content of essential amino acids is comprehensive and abundant, and the proportion of fresh and sweet amino acids in the flavor amino acids is relatively high. Correlation analysis was performed in combination with amino acid metabolism profiling and transcriptome analysis to identify the main contributors of amino acid synthesis in black and white mulberry. The results showed HK, PGAM, ENO and PK the genes in the backbone of the amino acid biosynthesis pathway, which had a direct impact on the synthesis of various amino acids, and were the main genes for amino acid synthesis in mulberry fruit. Through this study, we identified the transcription factors (TFs) and other structural genes involved in the amino acid metabolism during maturation of black and white mulberries. This study elucidated the spatiotemporal accumulation of amino acids and their metabolites at different developmental stages of black and white mulberry, and elucidated the potential regulatory pathways of black and white mulberry during the maturation process. This study provides the basis for the development of amino acid nutritional supplements and mulberry functional food.

1 Introduction

Mulberry (Morus alba), belongs to the genus Moraceae, and possess oval-shaped aggregate berries that are rich in nutrients, sweet and soft, and have a unique flavor[1] Mulberry is usually eaten as fresh fruit, jam and juice. They contain a large number of biologically active ingredients that may be associated with some potential pharmacological activities[2]. Epidemiological studies have shown that mulberry contains rich and effective chemical components with wide range of biological activities, such as antioxidant, anti-inflammatory, anti-cancer[3, 4]. The pharmacological effect of the mulberry fruits is attributed by the presence of various metabolites, particularly amino acids, flavonoids, and other secondary metabolites. Metabolomic profiling has revealed that the accumulation of specific metabolites, such as amino acids, is linked with the developmental stages of mulberry fruits[5, 6]. Comparison of metabolic profile between the diploid and tetraploid Morus alba, revealed that the tetraploid cultivar exhibited enhanced metabolic profiles of primary and secondary metabolites[7].

People choose food subjectively to meet the nutritional needs of the body. In recent years, with the deepening of people's understanding of food nutrition, foods rich in amino acids have attracted much attention[8]. Proteins are essential active macromolecules in living organisms, including enzymes that make up organs and catalyze metabolism[9]. Amino acids are the basic units of protein. Supplementation with essential amino acids that cannot be synthesized by the human body has a positive effect on maintaining the stability of the body and the progress of complex enzymatic

reactions[10]. These essential amino acids must be obtained through food[11]. Therefore, attention should be paid to the amino acid composition of food to maintain the body's demand for amino acids[12]. The eight essential amino acids include leucine, isoleucine, valine, lysine, threonine, methionine, phenylalanine, and tryptophan[13]. Phenylalanine is an important raw material for the biosynthesis of tyrosine, and is also the main component of some amino acid drugs and nutritional foods. In addition, it is also an intermediate for synthesizing the anticancer drug amphetamine[14]. Tryptophan can promote the secretion of melatonin, which plays an important role in regulating the body clock and improving sleep quality[15]. Studies have shown that glutamate is not only an important unit of protein but also the most abundant and widely distributed excitatory neurotransmitter in the central nervous system, which plays a key role in brain information processing and cognitive learning[16]. In addition, threonine and lysine are widely involved in human life activities. For example, threonine is a key amino acid for the synthesis of mucosal proteins, and lysine can promote intestinal absorption and utilization and enhance the function of immune system[17]. In addition, amino acids can be divided into sweet amino acids, umami amino acids, bitter amino acids, and astringent amino acids according to different flavors[11, 18].

Mulberry fruits and leaves serve as a rich source of amino acids, comprising of all nine essential amino acids that the human body is unable to synthesize on its own[19]. The balanced composition of essential amino acids in mulberry underscores its high nutritional value[20]. Recognized as a functional food, mulberry offers multiple health benefits which includes supporting digestion, lowering cholesterol levels, aiding in weight management, enhancing blood circulation, promoting bone tissue development, and strengthening the immune system[21, 22]. The composition of amino acids can vary significantly among different fruit types and ripening stages, influencing not only taste but also the nutritional value of the fruit. Thus, its mandatory to have the idea about the novel amino acid contents present in our food. Integration analysis of transcriptomic and metabolomic data have proven to be a powerful tool for elucidating the biosynthetic pathways of bioactive compounds synthesis and also aids in identifying the key metabolite pathways that leads to the discovery of novel compounds with potential health benefits.

In this study, we used metabolomic and transcriptomic approach to investigate the key genes and other regulatory mechanism behind amino acid biosynthesis in mulberry. The type and content of amino acids significantly influence the taste and nutrition of mulberry. However, limited information is available on the composition, quantity, andbiosynthesis of amino acids in mulberry fruits. With the recent advancement in analytical methods, multi-omics approaches have been widely used in the study of food components and their biological functions. In this study, we analyzed the amino acid biosynthetic pathways in black and white mulberry fruit and elucidated the role of amino acids in determining fruit quality, thereby providing a scientific basis for creating mulberry-based nutritional supplements.

2 Materials and Methods

2.1 Plants Materials

The black mulberry and white mulberry at different maturity stages required for the experiment were collected from the Chengde Medical College (Hebei, China).We confirm that the mature stage samples were collected from Chengde Medical University (Hebei Province, China), and we obtained official permission from the administration office of the university (the landowner). The fruit samples of black and white mulberry were collected from 7-year-old trees in the full-fruit period. The black (B) and white (W) mulberry were collected at three different growth periods: green fruit stage, turning stage, and maturation stage at 30, 60, and 90 days after flowering, respectively. Nine trees were selected for each period, and every-three trees served as a biological replicate, respectively. The samples were quickly cooled in liquid nitrogen and transferred to a -80℃freezer for amino acid metabolite determination and transcriptome sequencing.

2.2 Amino Acid Metabolite Extraction and UPLC-MS/MS Analysis

The collected samples were vacuum freeze-dried using a freeze dryer. After the sample was thawed and smashed, an amount of 0.05 g of the sample was mixed with 500 μL of 70% methanol/water. The sample was vortexed for 3 min under the condition of 2500 r/min and centrifuged at 12000 r/min for 10 min at 4°C. Take 300 μL of supernatant into a new centrifuge tube and place the supernatant in -20°C refrigerator for 30 min, Then the supernatant was centrifuged again at 12000 r/min for 10 min at 4°C. After centrifugation, transfer 200 μL of supernatant through Protein Precipitation Plate for further LC-MS analysis.

The sample extracts were analyzed using an LC-ESI-MS/MS system (UPLC, ExionLC AD, https://sciex.com.cn/; MS, QTRAP® 6500 + System, https://sciex.com/). The analytical conditions were as follows, HPLC: column, ACQUITY BEH Amide (i.d.2.1×100 mm, 1.7 μm); solvent system, water with 2 mM ammonium acetate and 0.04% formic acid (A), acetonitrile with 2 mM ammonium acetate and 0.04% formic acid (B); The gradient was started at 90% B (0-1.2 min), decreased to 60% B (9 min), 40% B (10–11 min), finaly ramped back to 90% B (11.01-15 min); flow rate, 0.4 mL/min; temperature, 40°C; injection volume: 2 μL. Amino acid and its metabolites were detected by MetWare (http://www.metware.cn/) based on the AB Sciex QTRAP 6500 LC-MS/MS platform.

2.3 Transcriptome Sequencing

The total RNA of black and white mulberry fruits was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's procedure. The concentration and purity of total RNA were quantified using NanoDrop ND-1000 (NanoDrop, Wilmington, DE, USA) and Bioanalyzer 2100 (Agilent, CA, USA) with RIN number > 7.0 to ensure the stability and accuracy of transcriptome sequencing library construction. The high-quality total RNA was sent to LC-Bio Technologies (Hangzhou) Co., Ltd. for libary construction and sequencing. Each biological replicate established a transcriptome library, a total of 18 libraries, and sequenced and analyzed using Illumina Novaseq™ 6000 (LC-Bio Technology CO., Ltd., Hangzhou, China) following the vendor's recommended protocol[1].

2.4 Principal Component Analysis

The relative levels of amino acids and their metabolites detected in black and white mulberry fruits and the expression levels of genes in the transcriptome were analyzed using the Omicshare data processing platform developed by LC-Bio Technologies (Hangzhou) Co., Ltd (https://www.omicshare.com/) for principal component analysis (PCA) analysis. The data was unit variance scaled before unsupervised PCA. The parameters were set by default.

2.5 Functional Annotation and Differentially Expressed Gene Analysis

The gene sequences were aligned in the Pfam database using HMMER software, and gene function annotation was performed. Fragments per kilobase of transcripts per million mapped fragments (FPKM) represented gene expression levels and were calculated using the read count of unigenes. The differentially expressed mRNAs were selected with fold change > 2 or fold change < 0.5 and p value < 0.05 by DESeq R software package [2]. The sequences obtained by sequencing were compared based on GO and KEGG databases and analyzed using BLAST software.

2.6 Gene Expression Trend Analysis

All differentially expressed genes of black and white mulberry fruits in different maturity stages were extracted, and the FPKM value was used to represent the gene expression level for gene expression trend analysis. The gene expression level data were imported into STEM software foranalysis. The gene expression level data were normalized using log2(FPKM), the clustering method was K-mean.

2.7 Weighted Gene Co-expression Network Analysis (WGCNA)

WGCNA was performed in R using default parameters to cluster genes into co-expressed modules[3]. The FPKM values were normalized, and an adjacency matrix was constructed. Metabolites data were imported into the WGCNA package, and correlation-based associations between metabolites and gene modules were calculated using the default settings. A rigorous multiple test correction ($r \geq 0.9$ or $r \leq -0.9$) was used to fflter the metabolites, TFs and structural genes that were signiffcantly correlated with each metabolite. The interaction networks were visualized using cytoscape (version 3.7.2).

3 Results

3.1 Amino Acid and their Metabolites Profile of Black and White Mulberry Fruits

The analysis of amino acids and their metabolites at different developmental stages of black and white mulberry fruits revealed the presence of various amino acids and their derivatives including two umami amino acids and six sweet amino acids (Additional file 1). PCA showed that principal component 1 (PC1) and principal component 2 (PC2) together accounted for 98.91% of the total variance, with PC1

contributing 96.82% and PC2 contributing 2.09% (Fig. 1A). PCA and intragroup correlation reflected the clear differences between sample groups and high similarity among biological replicates (Fig. 1B). The intragroup correlation analysis of black and white mulberry fruits at different developmental stages indicated significant differences in amino acid profiles, while high correlation among biological replicates and also met the requirements of data analysis. In total, 65 differentially accumulated metabolites (DAM) were identified between different developmental stages (Additional file 2). Specifically, 12 DAM between B1 and W1 (11 down-regulated/1 up-regulated), 16 DAM between B2 and W2 (9 down-regulated/7 up-regulated), and 11 DAM between B3 and W3 (5 down-regulated/6 up-regulated) (Fig. 1C). Additionally, there were 31 DAM between W3 and W1 (10 down-regulated/21 up-regulated), and 24 DAM between B3 and B1 (12 down-regulated/12 up-regulated). The number of DAM in each comparison indicated that the amino acids accumulated profiles were significantly differ with different cultivars and the DAM was higher in the W3 indicating substantial metabolic shifts in this group (Fig. 1C). Data analysis showed that the proportion of fresh and sweet amino acids gradually increased during the ripening process of black and white mulberry, with higher accumulation at the developing stage when compared to the green fruit stage (Fig. 1D; Additional file 3). The accumulation of sweet amino acids is higher in the white mulberry when compared with the black mulberry. Overall, the results demonstrated that mulberry fruit are rich in amino acids, including eight essential amino acids required by the human body, of which tryptophan and threonine were the most abundant amino acids (Additional file 1).

3.2 Metabolites Changes of Black and White Mulberry Fruits

To gain further insight into variation in amino acids biosynthesis at different developmental stages, 65 DAM were divided into nine distinct groups (group 1–9) using the k-means clustering algorithm (Fig. 2 and Additional file 4). The results showed that differential accumulation of amino acids is stage and tissue specific. Group 1 metabolites exhibited uniform accumulation across all stages (B1-B3, W1-W3). The main metabolite involved in these processes are N8-acetylspermidine, glutamate–cysteine, and L-cystine. Group 2 metabolites exhibited moderate fluctuations but maintained relatively stable levels throughout development, with amino acids like N-acetyl-L-glutamine and trans-4-hydroxy-L-proline. Group 3 metabolites showed elevated accumulation in the early stages and reduced levels in black stages, with compounds such as N-isovalerylglycine and L-theanine. Group 4, 5, and 8 metabolites increased during early-development (B1-B2, W1-W2) stage before declining and it includes oxidized glutathione, L-tryptophan, L-glutamate, L-valine, L-tyrosine, L-leucine, and L-serine. Group 6 maintained relatively constant levels with minor variation, containing sulfur-containing amino acids such as L-homocysteine and L-cysteine. Group 7 metabolites showed a marked decrease at the early white stage (B2) with compounds such as N-acetylaspartate and D-alanyl-D-alanine. Group 9 metabolites displayed overall stable expression across both colour types, suggesting common amino acids like L-tyrosine methyl ester and trimethylamine-N-oxide.

3.3 Transcriptome Sequencing and Differentially Expressed Gene Analysis

The PCA of transcriptomes from black and white mulberry fruits revealed that PC1 and PC2 accounted for 34.04% and 14.1% of the total variance, respectively (Fig. 3A). The PCA results showed that the gene expression patterns of black and white mulberry fruits were similar at the green fruit stage (B1 and W1), while the differences between the two cultivars were significant at turning stage and maturation stage. The PCA plot demonstrated a clear distinction between the samples from different developmental stages, and also between the black and white fruits indicating distinct gene expression pattern linked to fruit maturation and genotype-specific transcriptional regulation. Differentially expressed gene (DEG) analysis indicates dynamic transcriptional changes at different developmental stages. In black mulberry, the highest number of DEGs was observed in B2 vs B3 (6985 genes), followed by B1 vs B3 (5910 genes), while the lowest was observed between B1 vs B2 (4730 genes) (Fig. 3B). Similarly, in white mulberry, between W2 vs W3 it was about (8443 genes); while between W1 vs W3 (5884 genes) and W1 vs W2 (5711 genes) (Fig. 3B). Across all comparisons, the number of down-regulated genes was greater than up-regulated genes, suggesting that gene repression plays a dominant role during fruit maturation. Venn diagram analysis have shown the shared and unique differentially expressed genes (DEGs) among various developmental stages (Fig. 3C). In white mulberry, 716 DEGs were shared across the W1 vs W2, W2 vs W3, and W1 vs W3 stages. In case of black mulberry, 543 DEGs were found to be commonly expressed across the B1 vs B2, B2 vs B3, and B1 vs B3. Notably, 351 genes were commonly regulated between black and white mulberry across their corresponding developmental stages (B1 vs W1, B2 vs W2, and B3 vs W3), indicating the presence of a conserved set of core genes associated with fruit development and ripening.

3.4 Pathway Analysis of Differential Genes Enrichment

Pathway enrichment analysis for DEGs have shown that it follows distinct yet partially overlapping metabolic pathways during mulberry fruit development (Fig. 4A–C). In green fruit stage (B1 Vs W1) (Fig. 4A), significant enrichment was observed in α-linolenic acid metabolism, protein processing in endoplasmic reticulum, glycerolipid metabolism, and plant hormone signal transduction, other types of O-glycan biosynthesis, glutathione metabolism, and starch and sucrose metabolism. In turning stage (B2 Vs W2) (Fig. 4B), enriched pathways included alpha-Linolenic acid metabolism, Photosynthesis-antenna proteins, Photosynthesis, Peroxisome, and Carbon fixation in photosynthetic organisms, highlighting lysine biosynthesis, pyruvate metabolism, cysteine and methionine metabolism, indicating enhanced photosynthetic activity and redox regulation throughout turning stage. The comparative analysis between black and white maturation stage (B3 Vs W3) (Fig. 4C) reveals shared enrichment in flavonoid, phenylpropanoid, starch and sucrose, and glutathione metabolism pathways, suggesting conserved molecular processes involved in pigment biosynthesis, sugar accumulation, and oxidative balance in both cultivars.

3.5 K-means Clustering of DEGs

To investigate the molecular mechanism of mulberry fruit maturation, we used the K-means clustering method to cluster the differentially expressed genes (DEGs) expressed during the turning stage of black and white mulberry (Additional file 5). Nine distinct gene expression clusters were identified and GO enrichment analyses were performed for each cluster based on temporal expression patterns (Fig. 5A). Group 2 (522 genes) and Group 7 (462 genes) were the largest, showing major transcriptional changes across developmental stages, while smaller clusters such as Groups 4 and 8 exhibited stage-specific expression patterns. Among all the 9 clusters, only 4 clusters (1, 4, 5, and 8) exhibit the pathways associated with amino acids and their metabolites. GO enrichment analysis (Fig. 5B, Additional file 6) revealed that Clusters 1 and 4 were enriched in serine/threonine kinase activity, transferase activity, and carbohydrate biosynthesis, whereas Clusters 5 was associated with glycolytic and oxidative metabolic processes. GO enrichment analysis showed that Group 5 genes were involved in “S-formylglutathione hydrolase activity” process; Group 8 genes are involved in “glycine catabolic process” (Fig. 5B).

Analysis of TF families in different clusters showed that they had different sensitivities for the effect of amino acid metabolism in mulberry. Transcription factor distribution (Fig. 5C) showed that MYB, bHLH, and WRKY families were predominant in Clusters 2, 3, and 7, while C2H2 and ERF TFs were enriched in Clusters 5 and 6. Overall, TFs were strongly represented in cluster 2 (69 TFs, 13.2% of DEGs), which includes six bHLH (XP_010093338.1, XP_010093617.1, XP_010099295.1, XP_010111900.1, XP_010107204.1 and XP_010099295.1), six C2H2 (XP_010094455.1, XP_010112909.1, XP_010097054.1, XP_010094455.1, XP_010099839.1 and XP_010089160.1), six Trihelix (XP_010095991.1 and five XP_010089783.1), five ARF (XP_010100721.1, XP_010100039.1, XP_010099050.1 and two XP_010106165.1), five MYB_related (XP_010098248.1, XP_010102082.1 and three XP_010109475.1), five NAC (two XP_010108843.1 and three XP_010091280.1), four C3H (XP_010102543.1, XP_010103154.1, XP_010099056.1, XP_010111195.1), four LBD (XP_010108453.1 and three XP_010107394.1), three MYB (XP_010086809.1, XP_010105966.1 and XP_010102234.1) and three TCP (XP_010097842.1, XP_010087414.1 and XP_010097842.1) (Additional file 7).

3.6 Co-expression Analysis and Identification of Genes in Response to Specific Biosynthesis by WGCNA

To investigate the relationships between the transcriptome and metabolome, we established a co-expression network analysis utilizing WGCNA to correlate 13 DAMs amino acids (Fig. 6). In addition, a co-expression network analysis was performed between different transcription factor families associated with the biosynthesis of specific amino acids. Module A, mainly consists of branched-chain amino acids such as valine, leucine, and isoleucine were predominantly associated with transcription factor families including bHLH, NAC, LBD, MYB-related, and ERF (Fig. 6A). Module B, consists of amino acids such as alanine, arginine, glutamate, and aspartate showed strong interactions with bHLH, GATA, AP2, NAC, NF-YA, and MYB-related transcription factors (Fig. 6B). Module C depicted a network cantered

around glutamine, glutamic acid, aspartate, alanine, and glutathione, with enriched associations with WRKY, bZIP, NF-YB, GRAS, AP2, and NAC families (Fig. 6C). Module D showed aromatic amino acids tryptophan, tyrosine, and phenylalanine as central metabolites linked to several transcription factors such as HD-ZIP and CPP, along with multiple gene loci (Fig. 6D). The analysis highlights that different transcription factor families have unique roles in amino acid metabolism in mulberry.

3.7 Mapping Gene Expression Pattern with Amino Acid Metabolic Pathway in Black and White Mulberry

To analyze the correlation between the amino acid content of mulberry fruits and its related gene expression, we mapped the amino acid synthesis pathway with the transcriptome data we obtained and based on that 23 genes were screened related to amino acid synthesis (Fig. 7). The heat map showed that the amino acid synthesis–related genes had different expression patterns during fruit development. The expression pattern reveals the dynamic regulation of genes involved in glycolysis, the TCA cycle, and the shikimate pathway during mulberry fruit development. The key glycolytic genes hexokinase (HK), phosphoglycerate mutase (PGAM), enolase (ENO), and pyruvate kinase (PK) showed increased expression at later stages (B3, W3), indicating enhanced carbon flux towards pyruvate. Within the TCA cycle, citrate synthase (CS), succinate-CoA synthetase and related genes displayed differential expression between black and white mulberries, implies that based on the genotype, variations occur in energy storage. The AST (Aspartate Aminotransferase) enzyme catalyses the transamination of oxaloacetate (OAA) to glutamate. Upregulation of aspartate serves as a precursor for the synthesis of asparagine and other amino acids. Genes associated with nitrogen metabolism such as glutamate dehydrogenase (GDH), asparagine synthetase (AS), and glutamine synthetase (GS) were upregulated during maturation, suggesting active amino acid interconversion (Fig. 7). Shikimate pathway genes 3-dehydroquinate dehydratase/shikimate dehydrogenase (DHQ/SDH), chorismate synthase (CSI), chorismate mutase (CM), and prephenate dehydratase (PDT) exhibited strong expression at B3 and W3, supporting elevated synthesis of aromatic amino acids (phenylalanine, tyrosine, tryptophan). Overall, coordinated activation of these pathways indicates enhanced amino acid and secondary metabolite biosynthesis during fruit maturation.

4 Discussion

Black and white mulberry fruits are recognized for their high nutritional value, and the nutritional profile of both have differences as black mulberry is rich in certain antioxidants while white mulberries are rich in vitamin C[4]. Mulberries contain a moderate level of amino acids that includes essential amino acids which are important for human health. The presence of amino acids contributes to the overall enhancement of nutrition and taste of the fruits[5]. The protein content of white mulberries is higher when compared to black ones[6]. Metabolite analysis has shown that white mulberry accumulates higher level sweet amino acids when compared to black ones. The accumulation of specific amino acids during fruit development and ripening is crucial for enhancing fruit quality attributes such as sweetness,

aroma, and texture. Comparative study between two species of strawberries found that higher concentrations of free amino acids is correlated with fruit quality attributes, such as taste and aroma[7]. Asparagine and glutamine serve as nitrogen sources that are integral to the metabolic processes in citrus fruits, influencing their growth and flavor characteristics[8]. Furthermore, the nutritional content of fruits, including amino acids, is increasingly recognized as a factor driving consumer preferences, as higher amino acid content is associated with enhanced health benefits and sensory qualities[9]. Variations in the levels of amino acids such as alanine and valine is correlated with the changes in aroma of the fruits[10]. Certain amino acids improve the sweetness of fruits, where asparagine increases the sweetness of the peach fruits[11].

4.1 Changes in Metabolite Accumulation during Fruit Maturation

As the fruit matures, amino acids and its related metabolite level get increased or decreased. The up and down-regulated metabolites have specific functions related to fruit maturation and overall quality. In both black and white mulberry, the metabolite N8-acetylspermidine, (a derivative of polyamine spermidine), has been found to get increased gradually from greening to maturation stage. The total sugar content in jujube fruit gets increased during ripening stage and is accompanied with increase in N8-acetylspermidine[12]. Further N8-acetylspermidine helps in fruit ripening by degradation of pectic polysaccharides[13] and by modulating various metabolic pathways. Apart from upregulated metabolites (group 1) there is a consistent decline in the group 4 metabolites (L-Tryptophan, Succinic Acid) during fruit maturation (Fig. 2). L-Tryptophan, serves as a precursor for auxin biosynthesis which gets declined during fruit maturation[14, 15]. At the time of fruit maturation, the metabolic energy flux may shift toward the synthesis of other important compounds, such as flavonoids and defensive metabolites, which contribute to fruit quality[16, 17]. The decline of succinic acid, indicates a reduced reliance on the TCA cycle for energy production. Succinic acid is a key intermediate in the energy producing TCA cycle. During fruit ripening, metabolic shift occurs where the decrese in the levels of various organic acids, including succinic acid, was correlated with the changes in metabolic activity and energy production[16]. This decline may shift the fruit's metabolic priorities, moving away from energy production toward the synthesis of compounds that contribute to fruit quality. Rise in respiration is associated with increased ethylene production, which triggers various ripening processes, including the breakdown of organic acids[18]. As respiration rates change, the metabolic pathways involved in energy production and organic acid metabolism are also affected, leading to a decline in succinic acid levels. This metabolic reprogramming can lead to reduced synthesis or increased degradation of S-sulfo-L-cysteine. The decline reflects that there is a decreased need for sulfur-containing compounds as fruit prioritizes other metabolic pathways that are critical for ripening. L-pipecolic acid, an amino acid derivative, is involved in plant defense mechanisms. As fruits ripen, the demand for certain metabolites associated with growth and defense may diminish, leading to a reduction in L-pipecolic acid levels. The decline in both S-sulfo-L-cysteine and L-pipecolic acid during fruit maturation reflects increased respiration rates, shifts in energy metabolism, and the synthesis of secondary metabolites that

contribute to fruit flavor, aroma, and color. Group 8 metabolites, (L-Lysine,L-methionine, Beta-alanine) accumulation got increased during the maturation stage. L-lysine is the precursor for the synthesis of various compounds, including proteins and secondary metabolites that contribute to flavor and aroma [19]. Degradation of L-methionine is essential for the formation of sulfur-containing volatiles that contribute to the aroma of melon fruit[20].

4.2 Key Genes Involved in the Fruit Maturation

During the fruit ripening stage, the role of ATP binding, glycolytic processes, carbohydrate transport, and sugar membrane transport become increasingly important which have specific roles to improve the overall fruit quality, flavor, and texture. ATP, as a universal energy currency, is essential for numerous biochemical processes, including those involved in fruit maturation. ATP binding is essential for energy transfer and metabolic regulation during the turning stage of fruit maturation. As fruits transition from the immature to the mature state, there is an increased demand for energy to support various biochemical processes, including cell wall modification and the synthesis of flavor compounds where ATP provides the energy. In peaches, it was shown that ATP is crucial for the activity of enzymes involved in ethylene production, which is a key hormone that regulates ripening[21]. Increase in ATP levels during the maturation of fruits like mango and papaya, indicates a correlation between ATP availability and the physiological processes that accompany fruit ripening[22, 23]. The increase in ATP levels during this stage is associated with enhanced metabolic activity, facilitating the conversion of starches to sugars, which is vital for flavor development [24].The glycolytic process plays a crucial role in fruit maturation, particularly during the turning stage, where significant biochemical changes occur as fruits transition from immature to mature states. As fruits mature, glycolysis is upregulated, leading to an increase in the levels of sugars such as glucose and fructose, which are critical for the sweetness and overall flavor profile of the fruit[24]. In peaches, the activity of glycolytic enzymes increases significantly during maturation, contributing to the accumulation of soluble sugars and organic acids that define fruit quality[25]. Apart from energy production they also provide intermediates that serve as precursors for other biosynthetic pathways. In citrus fruits, the upregulation of glycolytic enzymes during fruit maturation is linked to the synthesis of precursors for amino acid and fatty acid biosynthesis, contributing to the flavor and nutritional quality of the fruit[26]. In bananas, the expression of glycolytic genes is closely associated with the metabolism of starch and sucrose, indicating a direct link between glycolytic activity and the accumulation of sugars that enhance fruit sweetness[27]. Carbohydrate transport is vital for the distribution of sugars from source tissues (like leaves) to developing fruits. During the turning stage of the fruits, the transport of carbohydrates to the fruits occur to accumulate sugars. Transport proteins, particularly those in the ATP-binding cassette (ABC) family, facilitate the unloading of sucrose and other sugars into the fruit cells [28]. It is reported that in pomegranate fruits, the transport of carbohydrates is closely linked to the accumulation of total soluble solids (TSS), which increases during the fruit ripening stage[24]. This accumulation is essential for achieving the desired sweetness and flavor characteristics. Enoyl-(acyl-carrier-protein) reductase (ENR) and acetyl-CoA carboxylase (ACC) are two critical enzymes involved in fatty acid metabolism, which plays a significant role in fruit maturation. ENR catalyzes the final reduction step in the fatty acid elongation cycle,

converting trans-2-enoyl-ACP to acyl-ACP using NAD(P)H as a cofactor. This reaction is crucial for producing saturated fatty acids, which are integral components of membrane lipids and storage lipids in fruits [29, 30]. On the other hand, ACC catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, the first committed step in fatty acid biosynthesis[31]. This reaction is a key regulatory point in the fatty acid synthesis pathway, as malonyl-CoA serves as a building block for the elongation of fatty acid chains. The activity of ACC is tightly regulated during fruit maturation, as the demand for fatty acids increases for membrane formation and energy storage[32].

4.3 TFs Involvement during the Fruit Maturation

Co-expression network analysis shows that interaction occurred between branched-chain amino acids (BCAAs) such as isoleucine, valine, and leucine, and key transcription factors (TFs) including NAC, ERF, bHLH, and MYB-related proteins (Fig. 6A). Expression of certain TFs correlates with the accumulation of specific metabolites, such as sugars and organic acids, which are vital for fruit quality[33].

Transcription factors such as NAC, ERF, bHLH, and MYB are known to regulate various developmental processes in plants, including fruit ripening and stress responses[34, 25, 35]. In mulberry, the interplay between these amino acids and TFs NAC and MYB can influence fruit quality traits such as sugar and acid metabolism, which are critical for fruit flavor and nutritional value[36]. Co-expression networks analysis between NAC, G2-like, C2H2, and Trihelix transcription factors, and with amino acids such as alanine, arginine, glutamate, and aspartate shows that they have important roles as signaling molecules during stress responses. Correlation network studies have shown that Glutamate and NAC and C2H2 genes have strong coordination during stress responses[37]. Co-expression networks analysis between WRKY, bZIP, GRAS transcription factors, and with amino acids such as Glutamine, glutamic acid, aspartate, alanine, and glutathione shows their involvement in metabolic regulation. The expression of WRKY and bZIP transcription factors has been linked to the regulation of genes involved in the synthesis of GABA (gamma-aminobutyric acid), an abundant metabolite in mature fruits and improves the flavor of fruits[18]. GRAS family of transcription factors has been shown to interact with WRKY and bZIP factors, which enhances the fruits maturation processes[18]. Co-expression network analysis between HD-ZIP, CPP transcription factors, and with amino acids such as Tryptophan, tyrosine, and phenylalanine have specific roles in secondary metabolism. Tyrosine and phenylalanine are precursors for phenolic compounds and flavonoids, which contribute to the color, flavor, and antioxidant properties of fruits[38]. The co-expression of HD-ZIP transcription factors with these amino acids suggests a coordinated regulatory mechanism that enhances the production of these metabolites during fruit maturation.

4.4 The Biosynthetic Pathways of Flavor-related Amino Acids in White and Black Mulberry

Mulberry fruit flavor is closely linked to its amino acid metabolic pathways. Key amino acids—such as phenylalanine (Phe), tryptophan (Trp), glutamate (Glu), aspartate (Asp), and tyrosine (Tyr)-serve not only as direct flavor compounds or their precursors but are also precisely regulated by the differential

expression of key genes during fruit development. This intricate regulation results in significant flavor differences between white and black mulberry varieties.

Synthetic pathway analyses indicate that the differential gene expression between white (W) and black (B) mulberries is primarily concentrated during the W2/B2 and W3/B3 developmental stages of the fruit. For instance, across these three developmental periods, the gene expression of Chorismate Mutase (CM) and Prephenate Dehydratase (PDT) in the phenylalanine synthesis pathway is significantly downregulated in both mulberry types. Phenylalanine, is the precursor for anthocyanin synthesis, influences anthocyanin content, which in turn modulates sugar accumulation in the fruit, ultimately leading to differences in flavor profiles. Aspartate, an important umami amino acid, also exhibits regulated accumulation. During the W2/B2 and W3/B3 stages, the gene expression of Aspartate Aminotransferase (AST), Glutamate Dehydrogenase (GDH), and Glutamine Synthetase (GS) in black mulberries is significantly downregulated compared to white mulberries, leading to varietal differences in aspartate accumulation. For glutamate, the most potent natural umami amino acid, the gene expression of Asparagine Synthetase (AS) in black mulberries is significantly upregulated during the B2 stage, which enhances the umami perception in black mulberries. The metabolism of tryptophan, a precursor for the plant endogenous hormone auxin (Indole-3-acetic acid, IAA), is equally crucial. The significant downregulation of Tryptophan Synthase (TS) in black mulberries during the W2/B2 stage suggests that white mulberries might accumulate higher sugar content, as IAA regulates cell division and fruit expansion, indirectly affecting sugar accumulation and the distribution of flavor compounds. Tyrosine is a direct or indirect precursor for many volatile aromatic compounds (e.g., fruity, floral notes). The gene expression of Prephenate Dehydrogenase (PDH) in white mulberries is significantly downregulated relative to black mulberries, affecting the metabolic flux of tyrosine and consequently altering the fruit's aromatic profile. A more global metabolomic mapping analysis reveals that within the fundamental metabolic networks underpinning amino acid synthesis pathways—including glycolysis, the tricarboxylic acid (TCA) cycle, and the shikimate pathway—the gene expression in white mulberries is overall significantly upregulated compared to black mulberries. This indicates that within the same developmental timeframe, white mulberries can more efficiently drive carbon flux through these core metabolic pathways, thereby providing ample precursors and energy for the synthesis of more phenylalanine, tryptophan, glutamate, aspartate, and tyrosine.

In summary, the differential expression of key genes in the amino acid synthesis pathways between white and black mulberries profoundly influences the accumulation patterns of various flavor-related amino acids in each variety. These metabolic differences ultimately manifest in perceptible sensory characteristics: white mulberries are typically characterized by a honey-like sweetness, a sticky mouthfeel, high sugar content, and almost no sourness, whereas black mulberries present a sweet taste with a hint of acidity and a more complex flavor profileshould discuss the results and how they can be interpreted from the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

5 Conclusions

This study integrated amino acid metabolic profiling and transcriptome analysis to elucidate amino acid biosynthesis in black and white mulberries. White mulberries showed higher levels of sweet amino acids, while black mulberries exhibited a more balanced, mildly acidic flavor. 65 metabolites, including eight essential and eighteen flavor-related amino acids, were identified, with sweet and fresh amino acids abundant during the turning and maturation stages. The turning stage showed the highest number of differentially expressed genes, and clusters 2 and 5 were enriched for pathways related to sweet and sour amino acid metabolism. K-means clustering and enrichment analyses identified specific gene clusters (1, 4, 5, and 8) associated with amino acid metabolism, particularly those involved in the synthesis of sweet and sour amino acids. Fifteen key genes, particularly HK, PGAM, ENO, and PK, were identified as core regulators of amino acid biosynthesis. Mapping of metabolic process with transcriptome data show extensive transcriptional remodeling of amino acid biosynthetic pathways between black and white mulberry cultivars. The coordinated regulation of glycolysis, TCA cycle, and amino acid biosynthetic routes suggests complex metabolic networks that may underlie phenotypic differences in growth and nutritional composition of mulberry fruits. Further the coordinated activation of different pathways, along with the involvement of key transcription factor families, drives the dynamic accumulation of amino acids during fruit ripening. Future studies should correlate these gene expression patterns with metabolite profiling to validate the functional significance of observed transcriptional changes and elucidate the metabolic basis of cultivar-specific traits in mulberry.

Declarations

The manuscript has not been submitted for any academic award and contains no previously published material without proper citation. All listed authors have approved the submission.We confirm that the mature stage samples were collected from Chengde Medical University (Hebei Province, China), and we obtained official permission from the administration office of the university (the landowner).

Funding Declaration

This research was financially supported by the Hebei Province ‘333 Talent Project’ (A202101051); General Project of Department of Education of Hebei province (QN2020236); Natural Science Foundation of Hebei province (H2023406059, C2019406113).

Ethics approval: Not applicable

Consent to participate: Not applicable

Consent for publication: Not applicable

Clinical Trial Number: Not applicable

Acknowledgements: Not applicable.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/doi/s1, Additional file 1. Summary of the 85 detected metabolites with annotation. Additional file 2. Differentially accumulated metabolites (DAMs) of mulberry at three development stages. Additional file 3. Fresh and sweet amino acids of mulberry at three representative development stages. Additional file 4. Distribution of DAMs identified among different k-means clusters. Additional file 5. Distribution of the differentially expressed genes (DEGs) identified among different k-means clusters. Additional file 6. GO enrichment analysis for the DEGs in the nine clusters. Additional file 7. Heatmap distribution of transcription factor (TF) families in the nine clusters. Additional file 8. Key enzyme gene expression in amino acid biosynthesis and interconversion metabolic pathways.

Conflict of interests: All authors declared no conflicts of interest

Author Contributions: Zhichao Sun: Writing-review & editing, Methodology, Funding acquisition, Data curation, Conceptualization. Weizhen Zhang and Ruotong Zhang: Writing-original draft, Validation, Data curation, Visualization. R.M. Saravana Kumar: Formal analysis. Jisheng Li: Supervision, Resources.

Data availability:

The following supporting information can be downloaded at: https://www.mdpi.com/article/doi/s1.The datasets generated and/or analysed during the current study are available in the OMIX database, hosted by the National Genomics Data Center (NGDC), Beijing Institute of Genomics, Chinese Academy of Sciences, under accession number OMIX016227 and OMIX0016229. The repository can be found at: https://ngdc.cncb.ac.cn/omix/preview/QH0PcxUR and
https://ngdc.cncb.ac.cn/omix/preview/KKqm4UEy

References

1. Dunning MJ, Smith ML, Ritchie ME, Tavaré S. beadarray: R classes and methods for Illumina bead-based data. Bioinformatics. 2007;23(16):2183–4. 10.1093/bioinformatics/btm311.
2. Anders S, Huber W. Differential expression analysis for sequence count data. Genome Biol. 2010;11:R106.
3. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics. 2008;9:559. 10.1186/1471-2105-9-559.
4. Polumackanycz M, Wesolowski M, Viapiana A. Morus alba L. and Morus nigra L. Leaves as a Promising Food Source of Phenolic Compounds with Antioxidant Activity. Plant Foods Hum Nutr. 2021;76(4):458–65. 10.1007/s11130-021-00922-7.
5. Georges Makhoul* HM, Hussam Baroudi#. Some Chemical Characteristics of White (Morus Alba L) and Black (Morus Nigra L) Mulberry Phenotypes in Tartus Syria. Agric Environ Sci. 2017;4(2):54–63.
6. Skender A, Kurtovic M, Ercisli S, Becirspahic D. Some physicochemical characteristics of black and white mulberry genotypes from Bosnia and Herzegovina. Genetika. 2019;51(3):1089–101. 10.2298/gensr1903089s.

7. Shen J, Shao W, Li J, Lu H. Integrated metabolomic and transcriptomic analysis reveals factors underlying differences in fruit quality between Fragaria nilgerrensis and Fragaria pentaphylla. J Sci Food Agric. 2022;102(8):3287–96. 10.1002/jsfa.11674.
8. Kim SS, Kim HJ, Park KJ, Kang SB, Park Y, Han SG, et al. Metabolomic Profiling of Citrus unshiu during Different Stages of Fruit Development. Plants (Basel). 2022;11(7):967. 10.3390/plants11070967.
9. Xiong B, Li Q, Yao J, Zheng W, Ou Y, He Y, et al. Transcriptome and UPLC-MS/MS reveal mechanisms of amino acid biosynthesis in sweet orange 'Newhall' after different rootstocks grafting. Front Plant Sci. 2023;14:1216826. 10.3389/fpls.2023.1216826.
10. Pérez AG, Olías R, Luaces P, Sanz C. Biosynthesis of strawberry aroma compounds through amino acid metabolism. J Agric Food Chem. 2002;50(14):4037–42. 10.1021/jf011465r.
11. Wang YS, Chen XY, Li LP, Hu L. Effect of 1-MCP and Salicylic Acid on Sugars, Organic Acids and Amino Acids in Peach Fruit during Postharvest Ripening. Adv Mater Res. 2012;554–556:1361–6. 10.4028/www.scientific.net/AMR.554-556.1361.
12. Yan M, Wang Y, Watharkar RB, Pu Y, Wu C, Lin M, et al. Physicochemical and antioxidant activity of fruit harvested from eight jujube (Ziziphus jujuba Mill.) cultivars at different development stages. Sci Rep. 2022;12(1):2272. 10.1038/s41598-022-06313-5.
13. Airianah OB, Vreeburg RA, Fry SC. Pectic polysaccharides are attacked by hydroxyl radicals in ripening fruit: evidence from a fluorescent fingerprinting method. Ann Bot. 2016;117(3):441–55. 10.1093/aob/mcv192.
14. Wang A, Li R, Ren L, Gao X, Zhang Y, Ma Z, et al. A comparative metabolomics study of flavonoids in sweet potato with different flesh colors (Ipomoea batatas (L.) Lam). Food Chem. 2018;260:124–34. 10.1016/j.foodchem.2018.03.125.
15. Zhang H, Ma ZF, Luo X, Li X. Effects of Mulberry Fruit (Morus alba L.) Consumption on Health Outcomes: A Mini-Review. Antioxid (Basel). 2018;7(5):69. 10.3390/antiox7050069.
16. Patel MK, Mishra A, Jaiswar S, Jha B. Metabolic profiling and scavenging activities of developing circumscissile fruit of psyllium (Plantago ovata Forssk.) reveal variation in primary and secondary metabolites. BMC Plant Biol. 2020;20(1):116. 10.1186/s12870-020-2318-5.
17. Winkel-Shirley B. Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiol. 2001;126(2):485–93. 10.1104/pp.126.2.485.
18. Setiawan E. Amino Acids Content in Mangosteen Fruit as Affected by Tree Ages. Agroekoteknologi. 2017;10(1):33–8. 10.21107/agrovigor.v10i1.278.
19. Katz YS, Galili G, Amir R. Regulatory role of cystathionine-gamma-synthase and de novo synthesis of methionine in ethylene production during tomato fruit ripening. Plant Mol Biol. 2006;61(1–2):255–68. 10.1007/s11103-006-0009-8.
20. Gonda I, Lev S, Bar E, Sikron N, Portnoy V, Davidovich-Rikanati R, et al. Catabolism of L-methionine in the formation of sulfur and other volatiles in melon (Cucumis melo L.) fruit. Plant J. 2013;74(3):458–72. 10.1111/tpj.12149.

21. Camejo D, Martí MC, Román P, Ortiz A, Jiménez A. Antioxidant system and protein pattern in peach fruits at two maturation stages. J Agric Food Chem. 2010;58(20):11140–7. 10.1021/jf102807t.
22. Teh HF, Neoh BK, Hong MP, Low JY, Ng TL, Ithnin N, et al. Differential metabolite profiles during fruit development in high-yielding oil palm mesocarp. PLoS ONE. 2013;8(4):e61344. 10.1371/journal.pone.0061344.
23. Loei H, Lim J, Tan M, Lim TK, Lin QS, Chew FT, et al. Proteomic analysis of the oil palm fruit mesocarp reveals elevated oxidative phosphorylation activity is critical for increased storage oil production. J Proteome Res. 2013;12(11):5096–109. 10.1021/pr400606h.
24. Mphahlele RR, Caleb OJ, Fawole OA, Opara UL. Effects of different maturity stages and growing locations on changes in chemical, biochemical and aroma volatile composition of 'Wonderful' pomegranate juice. J Sci Food Agric. 2016;96(3):1002–9. 10.1002/jsfa.7186.
25. Zhang Y, Chen Y, Zhou Y, Zhang J, Bai H, Zheng C. Comparative Transcriptome Reveals the Genes' Adaption to Herkogamy of Lumnitzera littorea (Jack) Voigt. Front Genet. 2020;11:584817. 10.3389/fgene.2020.584817.
26. Yao L, Yu Q, Huang M, Hung W, Grosser J, Chen S, et al. Proteomic and metabolomic analyses provide insight into the off-flavour of fruits from citrus trees infected with 'Candidatus Liberibacter asiaticus'. Hortic Res. 2019;6:31. 10.1038/s41438-018-0109-z.
27. Xia Y, Chiu CH, Do YY, Huang PL. Expression Fluctuations of Genes Involved in Carbohydrate Metabolism Affected by Alterations of Ethylene Biosynthesis Associated with Ripening in Banana Fruit. Plants (Basel). 2020;9(9):1120. 10.3390/plants9091120.
28. Lievre DL, Anderson R, Boldingh H, Cooney J, Seelye R, Gould N, et al. Modifying Carbohydrate Supply to Fruit during Development Changes the Composition and Flavour of Actinidia chinensis var. chinensis 'Zesy002' Kiwifruit. Plants (Basel). 2021;10(7):1328. 10.3390/plants10071328.
29. Seol G, Park H, Ahn Y-J, Kang L-W. Crystal structure of enoyl-acyl carrier protein reductase (fabi) from acinetobacter baumannii as a target for broad-spectrum antibacterial drug. Bull Korean Chem Soc. 2019;40(10):1039–42. org/10.1002/bkcs.11861.
30. Mao YH, Li F, Luo LZ, Yin Y, Ma JC, Zhang WB, et al. The low enoyl-acyl carrier protein reductase activity of FabI2 is responsible for the high unsaturated fatty acid composition in Sinorhizobium meliloti. BMC Microbiol. 2024;24(1):517. 10.1186/s12866-024-03645-2.
31. Fazili IS, Masoodi M, Ahmad S, Jamal A, Khan JS, Abdin MZ. Oil biosynthesis and its related variables in developing seeds of mustard (Brassica juncea L.) as influenced by sulphur fertilization. Crop Sci Biotechnol. 2010;13(1):39–46. org/10.1007/s12892-009-0117-5.
32. Broussard TC, Price AE, Laborde SM, Waldrop GL. Complex formation and regulation of Escherichia coli acetyl-CoA carboxylase. Biochemistry. 2013;52(19):3346–57. 10.1021/bi4000707.
33. Jia H, Xu Y, Deng Y, Xie Y, Gao Z, Lang Z, et al. Key transcription factors regulate fruit ripening and metabolite accumulation in tomato. Plant Physiol. 2024;195(3):2256–73. 10.1093/plphys/kiae195.
34. Jiang C, Zhang H, Ren J, Dong J, Zhao X, Wang X, et al. Comparative Transcriptome-Based Mining and Expression Profiling of Transcription Factors Related to Cold Tolerance in Peanut. Int J Mol Sci.

2020;21(6):1921. 10.3390/ijms21061921.

35. Kang JN, Lee WH, Won SY, Chang S, Hong JP, Oh TJ, et al. Systemic Expression of Genes Involved in the Plant Defense Response Induced by Wounding in Senna tora. Int J Mol Sci. 2021;22(18):10073. 10.3390/ijms221810073.
36. Zhang J, Wen M, Dai R, Liu X, Wang C. Comparative Physiological and Transcriptome Analyses Reveal Mechanisms of Salicylic-Acid-Reduced Postharvest Ripening in 'Hosui' Pears (Pyrus pyrifolia Nakai). Plants (Basel). 2023;12(19):3429. 10.3390/plants12193429.
37. Gourcilleau D, Lenne C, Armenise C, Moulia B, Julien JL, Bronner G, et al. Phylogenetic study of plant Q-type C2H2 zinc finger proteins and expression analysis of poplar genes in response to osmotic, cold and mechanical stresses. DNA Res. 2011;18(2):77–92. 10.1093/dnares/dsr001.
38. Brandt R, Salla-Martret M, Bou-Torrent J, Musielak T, Stahl M, Lanz C, et al. Genome-wide binding-site analysis of REVOLUTA reveals a link between leaf patterning and light-mediated growth responses. Plant J. 2012;72(1):31–42. 10.1111/j.1365-313X.2012.05049.x.

Figures

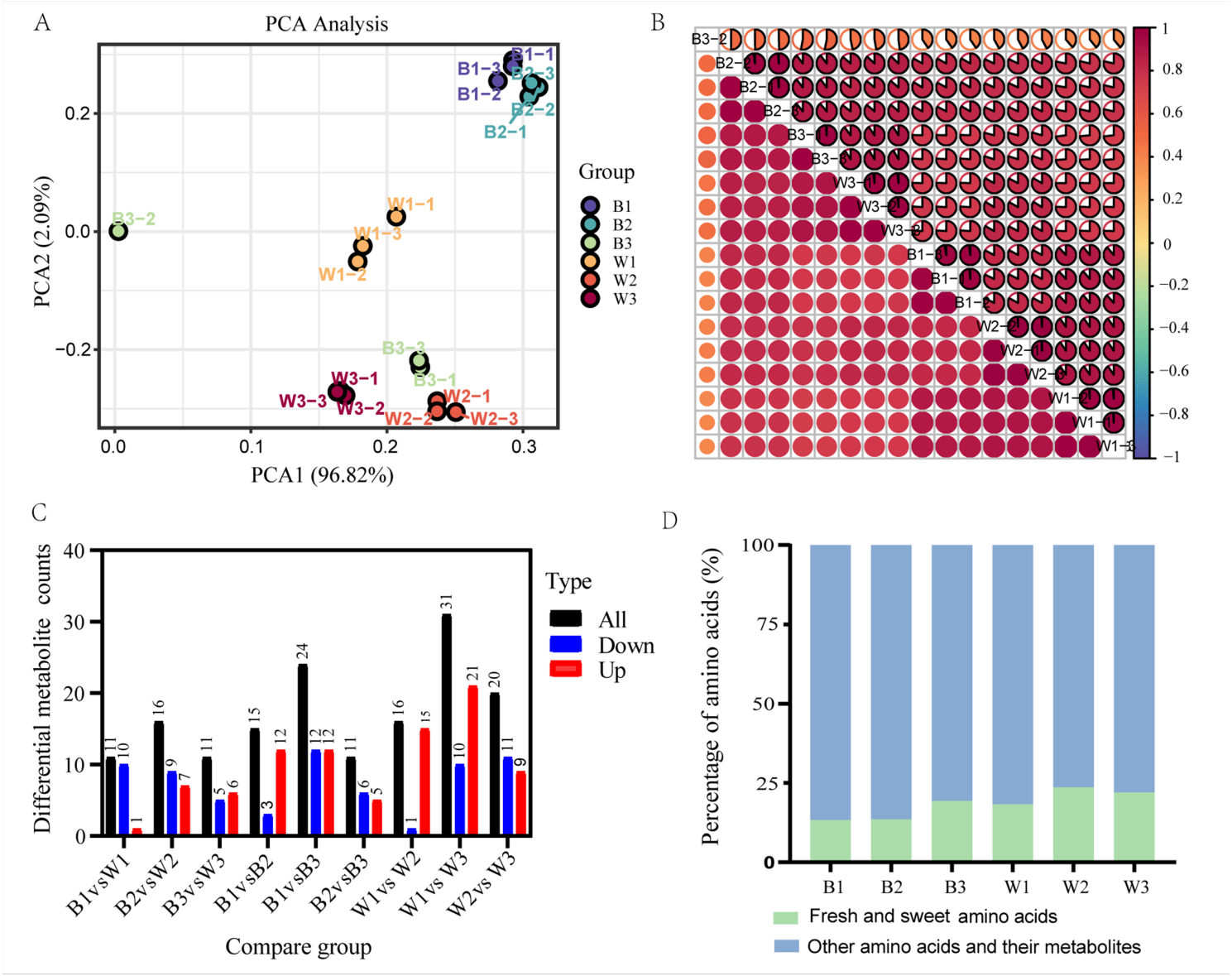


Figure 1

Amino Acid Metabolite Profile of Black and White Mulberry Fruits.(A) PCA analysis of amino acids at different developmental stages (Greening (B1, W1), turning (B2, W2) and maturation (B3,W3)). Where B stands for black and W stands for White mulberry fruits. (B) Intra-group correlation of black (B) and white (W) mulberry samples at different developmental stages. (C) Analysis of differential metabolite accumulation among different group of mulberry fruits. For black columns represent all differential metabolite counts; blue columns represent downregulated differential metabolite counts; red columns represent upregulated differential metabolite counts. (D) Ratio of fresh and sweet amino acids to total amino acids at different groups.

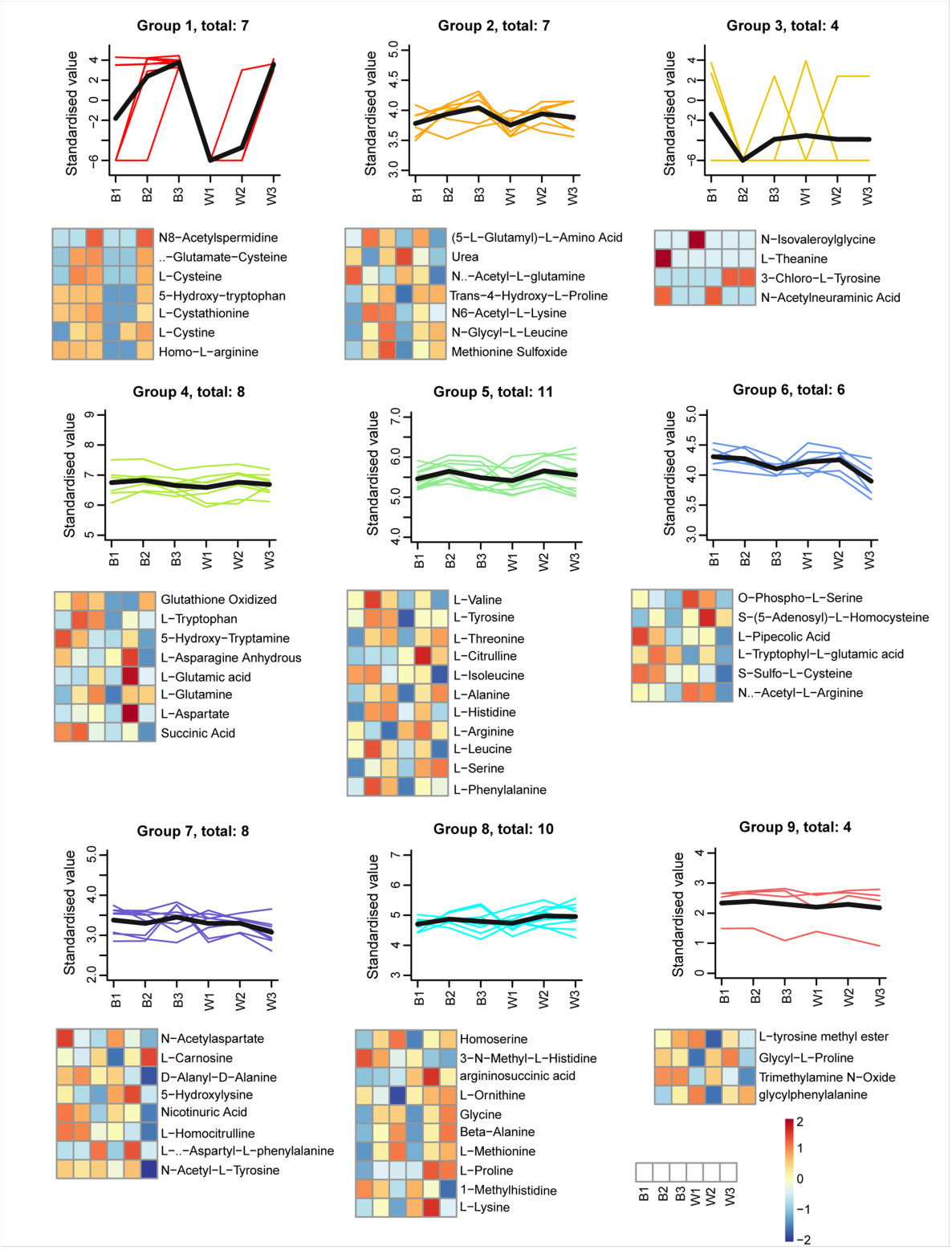


Figure 2

K-Means Cluster Analysis of 65 Amino Acids. A total of 65 amino acids and their metabolites were divided into nine groups using the k-means clustering algorithm. The x axis represents six key development stages (B1-B3, W1-W3) and the y axis depicts the standardised value metabolite content. The total number is expressed as the number of amino acids and their metabolites contained in the corresponding cluster (groups 1-9).

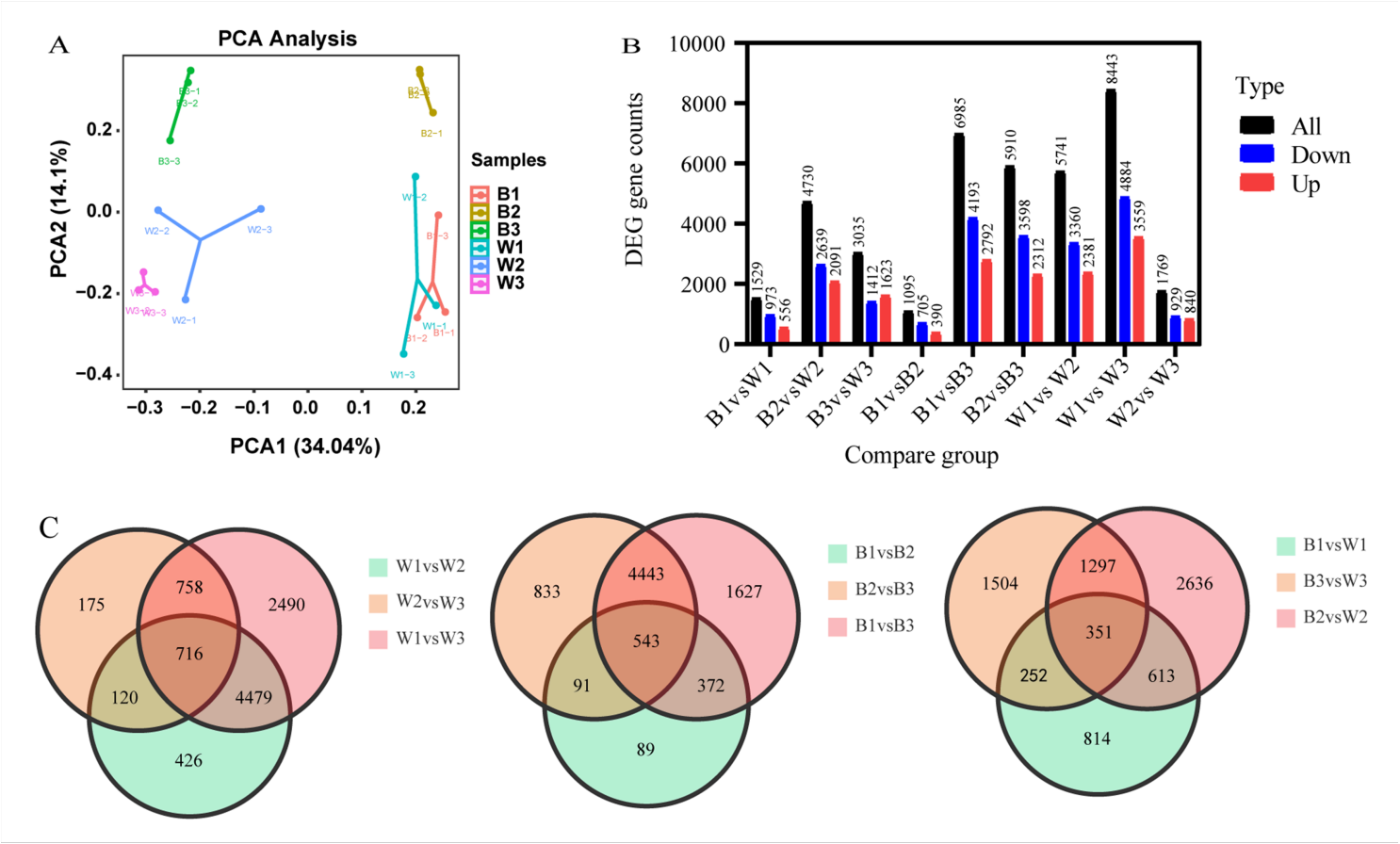


Figure 3

Transcriptome Sequencing and Differentially Expressed Genes in Black and White Mulberry Fruits. (A) PCA analysis of black and white mulberry transcriptomes. (B) Count of DEGs among each group of black and white mulberry fruits. For B vs W, black columns represent all DEGs; blue columns represent dpwnregulated DEGs; red columns represent upregulated DEGs. (C) Venn diagram of DEGs in the compared mulberry fruits groups. The number in each circle represents the number of DEGs expressed in this stage. Numbers in each overlapping part represent DEGs that were coexpressed in multiple stages.

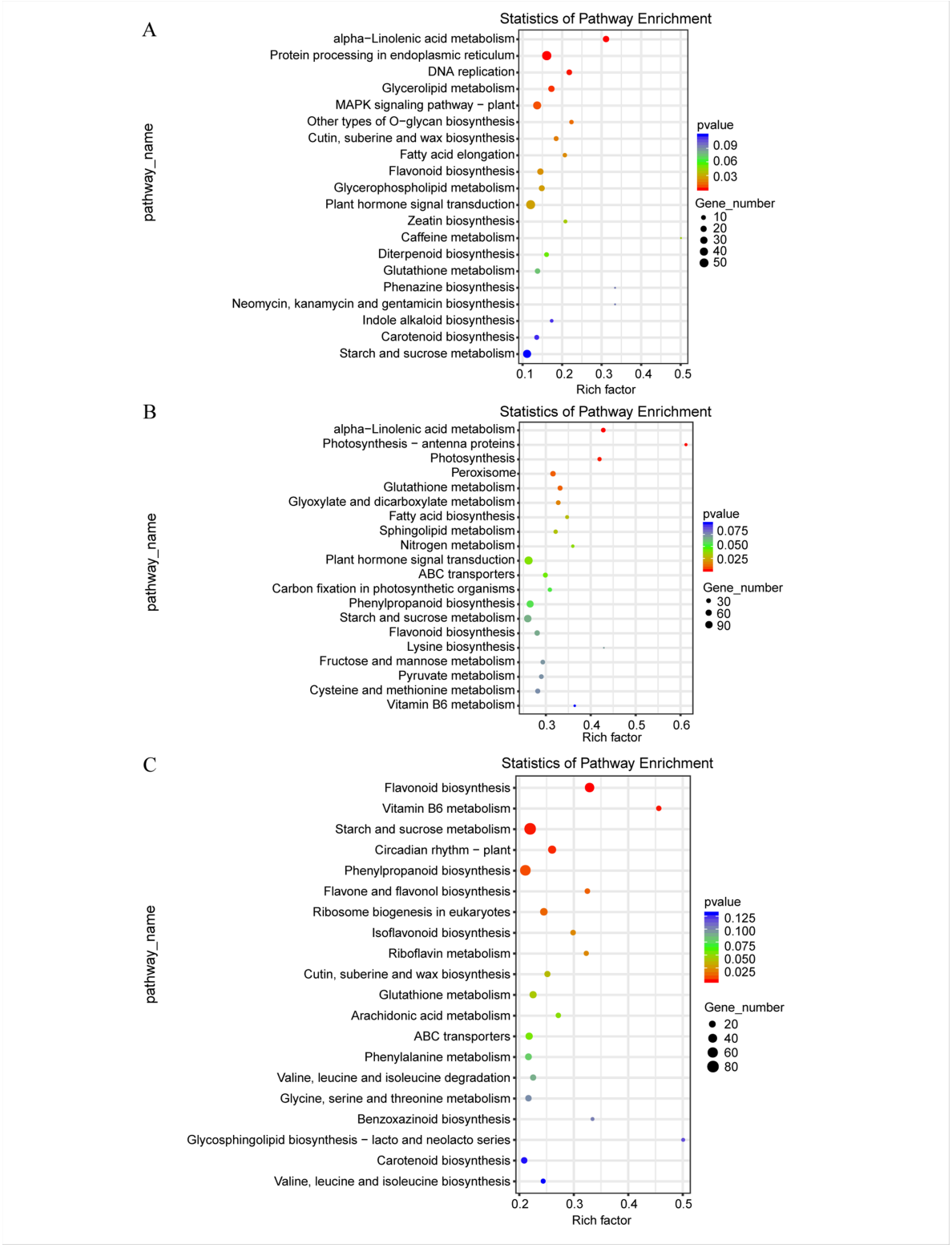


Figure 4

KEGG Pathway Enrichment Analysis at Three Developmental Stages. (A) KEGG pathway enrichment in green fruit stage (B1 Vs W1). (B) KEGG pathway enrichment in turning stage (B2 Vs W2). (C) KEGG pathway enrichment in maturation stage (B3 Vs W3). The size of the bubble indicates the number of amino acids and their metabolites in the pathway; the color range represents the significance of the pathway enrichment.

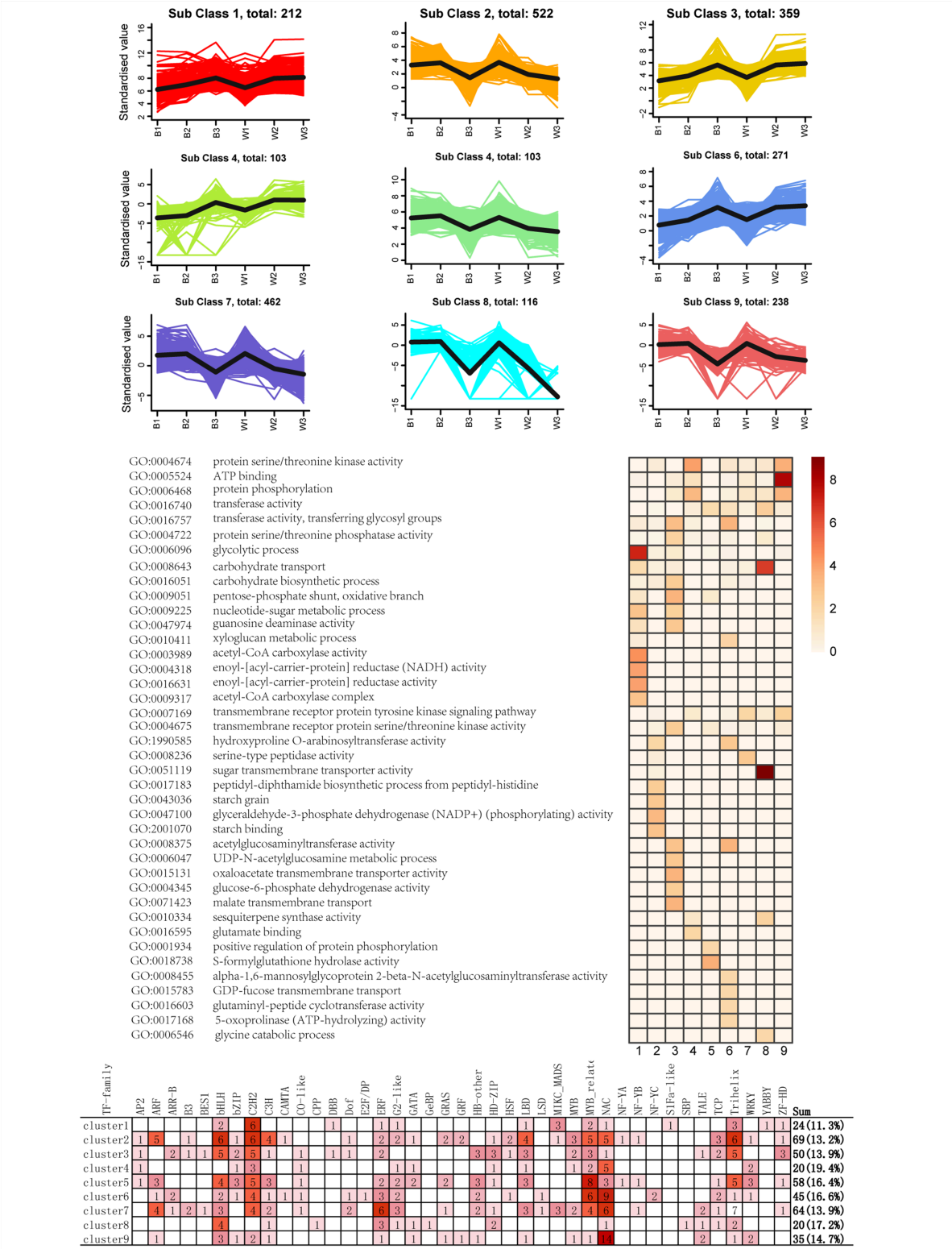


Figure 5

Cluster Analysis of Differentially Expressed Genes (DEGs) using K-means Clustering Method. (A) Nine clusters were identified by K-means clustering method. (B) GO enrichment analysis for the DEGs in the nine clusters. GO terms are listed alongside with associated biological functions, and heatmap illustrating the enrichment scores. (C) Heatmap distribution of transcription factor (TF) families in the nine clusters. Full list of TFs in the nine clusters were listed in Additional file 7.

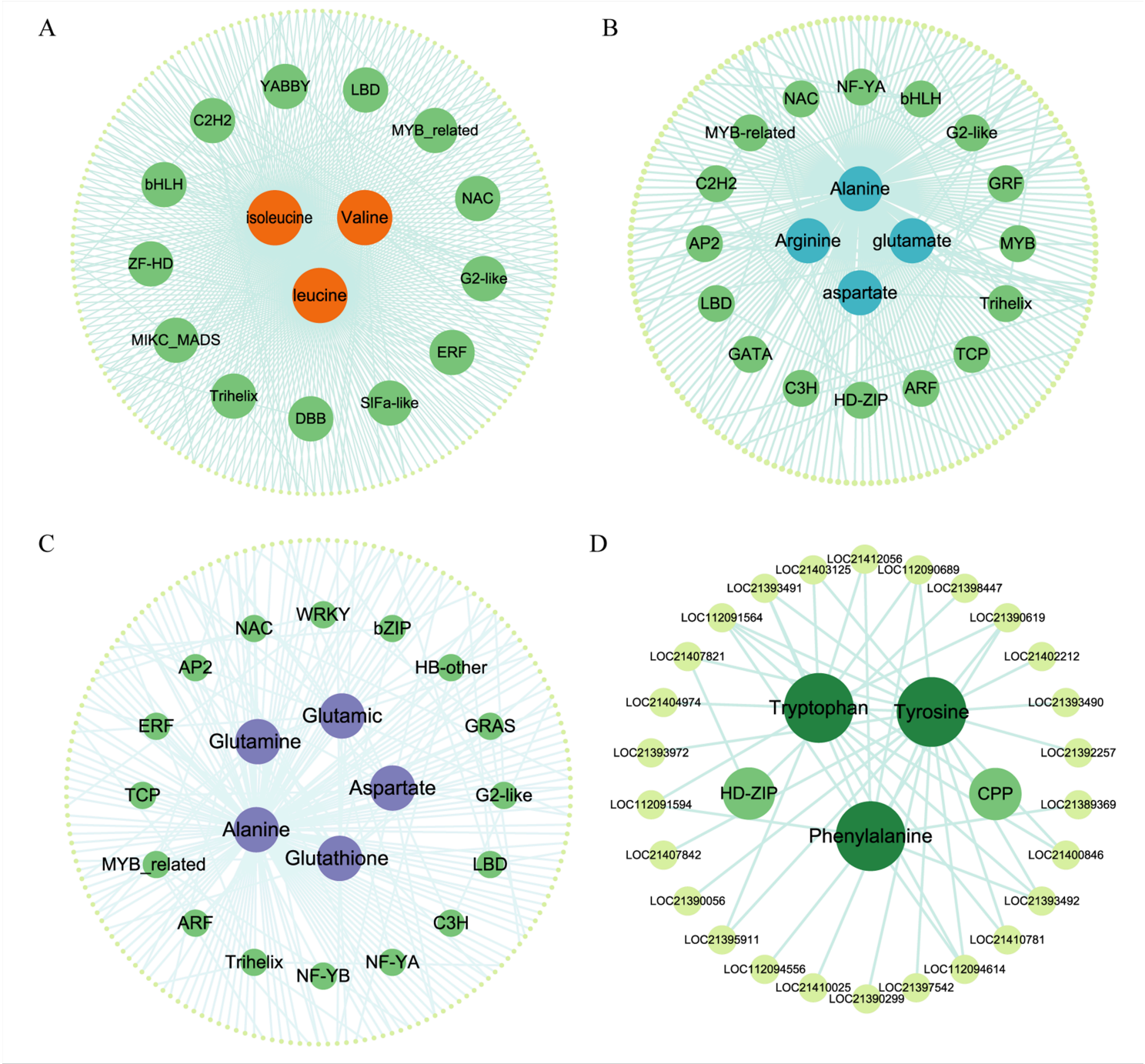


Figure 6

Co-expression Network Analysis between Amino Acids and Transcription Factors (TFs).

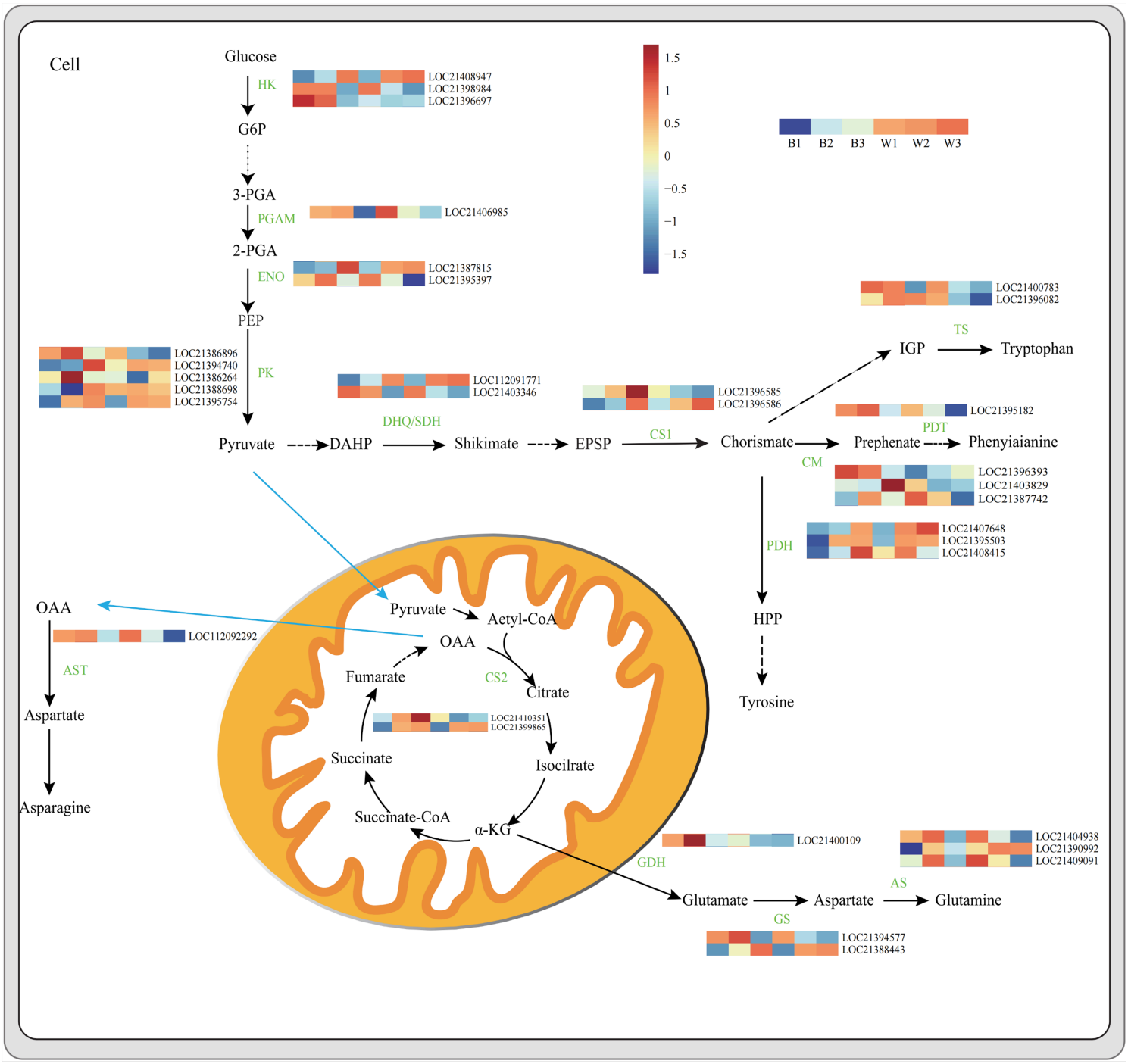


Figure 7

Schematic Illustration of Metabolic Pathways Involved in the Biosynthesis and Interconversion of Amino Acids in Black and White Mulberry. The green font in the diagram indicates key enzymes in the metabolic pathways. Arrows represent the direction of metabolite flow: solid arrows denote single-step reactions, dashed arrows represent multi-step reactions, and blue arrows indicate mitochondrial substrate shuttles. The names of enzymes and additional details are provided in Additional file 8. This pathway integrates central metabolic intermediates (such as glucose-6-phosphate, pyruvate, and oxaloacetate) and branches into specific amino acid biosynthesis routes, including tryptophan, aspartate, glutamate,

phenylalanine, and tyrosine. The color gradient scale in the heatmap, ranging from -1.5 to 1.5, represents the log10 fold change in gene expression differences.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile.zip