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COMPARATIVE ANALYSIS OF METABOLITES AND GENE EXPRESSION VARIABILITY IN SIX CONTRASTING POTATO VARIETIES: IMPLICATION FOR BREEDING AND NUTRITIONAL QUALITY

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ABSTRACT

As one of the four major staple crops, potato exhibits significant differences among varieties. Therefore, understanding the relationship between nutritional quality and molecular metabolism in potato varieties is essential for facilitating the development of the processing industry and improving dietary nutrition. In this study, six potato varieties: Mincaishu 1 (C1), Mincaishu 2 (C2), Mincaishu 3 (C3), Mincaishu 4 (C4), Minshu 2 (M2), and Minshu 3 (M3) were tested for metabolomics and transcriptomics analyses. The aim of this study was to investigate the molecular basis underlying the formation of distinctive traits in these varieties. The two varieties with the greatest differences in metabolites were C4 and C1, exhibiting disparities in 657 substances. These differential substances were primarily involved in metabolic pathways such as the synthesis and degradation of ketone bodies, the citrate cycle (TCA cycle), and alanine, aspartate, and glutamate metabolism. Further transcriptomic analysis revealed that the overall gene expression level in stems was lower than in leaves for all six varieties. However, the expression patterns of the same genes were consistent between leaves and stems across different varieties. The highest number of differentially expressed genes was observed in the leaves of C4 and C1. Additionally, through a correlation analysis of transcriptomic and metabolomic data, this study identified several shared pathways between the two omics approaches, including the citrate cycle and sulfur relay system metabolism. This study will serve as a cornerstone for advancing potato breeding and agricultural practices by improving yield, quality, and the metabolic profile of important potato traits, with our multi-omics analysis providing crucial insights into potato metabolism and facilitating the exploration of innovative breeding strategies.

Keywords: Potato, Metabolomics, Transcriptomics, Citrate cycle, Potato breeding.

INTRODUCTION

Potato (*Solanum tuberosum* L.), one of the world's most significant staple crops, provide essential nourishment and support for millions of people. Potatoes contain good quantities of basic nutrients such as carbohydrates, fiber, and vitamins C and B1, as well as minerals such as potassium and phosphorus. They also have a range of specialized metabolites that play an important role in several processes (Aversano *et al.*, 2017). Given the high demand for potatoes, there must be a continuous focus on increasing yields, maintaining quality, and enhancing tolerance to biotic and abiotic stresses (Ahmadu *et al.*, 2021). To achieve these aims, a full comprehension of the molecular and metabolic mechanisms behind the variance observed among many potato cultivars is necessary. The genetic makeup of individual potato varieties affects their physiological response, adaptability, and overall agronomic performance. Numerous factors, including the environment, nutrient availability, and genetic variation, impact the intricate interplay of genes and metabolites, ultimately defining the potato phenotype (Kumari *et al.*, 2018; Tessema *et al.*, 2020). A genome wise study performed by Hirsch *et al.* (2016) and Machida-Hirano and Niino (2017) suggested that the genome sequence information could be useful to identify novel alleles and genes absent in cultivated potato and may help explain Solanum species diversity, elucidating the differences in biochemical and molecular mechanisms leading to the formation of various specialized metabolites produced by wild and cultivated species. Another study performed by

Aversano *et al*. (2017) reported the results of metabolic HPLC-DAD and LC-ESI-MS analysis performed on tuber extracts from 31 clones of 13 Solanum species and from cultivated *S. tuberosum*, in order to investigate inter- and intra-specific differences in some human health-related metabolites that represent important breeding targets. They found higher levels of ascorbic acid and chlorogenic acid in their findings.

The cutting-edge scientific field of metabolomics aims to identify and quantify all small molecules or metabolites present in biological systems to address contemporary issues in modern society (Tsugawa *et al*., 2021). These metabolites serve as the culmination of an organism's metabolic activity since they show the outcomes of cellular processes. In parallel, transcriptomics investigates the dynamic patterns of gene expression, providing crucial information on the potential roles of discovered metabolites (Li *et al*., 2019).

An integrated approach may provide a better understanding of the molecular mechanisms underlying characteristics, such as tuber production, nutritional content, disease resistance, and environmental adaptability. Additionally, this research may identify novel candidate genes and important metabolic pathways associated with the desired traits, enabling targeted breeding strategies for developing superior potato cultivars (Boutsika *et al*., 2022). Increased nutritional content, disease resistance, and climate adaptability in potato cultivars may greatly enhance agricultural sustainability and global food security (Pixley *et al*., 2022). For this study, a total of 6 different potato varieties have been selected. By combining diverse omics data, we will be able to comprehend intricate molecular networks and discover the probable relationships between genes and metabolites. In a recent similar study, approximately 200 potato species from southwestern United States were selected for their broader genetic basis and metabolic contents, which were used for designing the appropriate breeding strategies for potato biofortification (Bradshaw, 2021). In the current study, we hypothesized to identify the genetic markers underlying the observed phenotypic variations by comparing the expression of metabolites and various genes in different potato cultivars.

MATERIALS AND METHODS

Sample collection and preparation: Six potato varieties, including Mincaishu 1, Mincaishu 2, Mincaishu 3, Mincaishu 4, Minshu 2, and Minshu 3, were selected as test materials and designated as C1, C2, C3, C3, M2, and M3, respectively. The six potato varieties were grown at the Potato Experimental Demonstration Base in Nanping Village, Shajiang Town, Xiapu County, Fujian Province, China. Samples were collected during the tuber bulking period with six biological replicates for each of the six varieties, resulting in a total of 36 samples. The collected samples were rapidly frozen in liquid nitrogen and stored at -80°C for subsequent metabolomic analysis. The leaves and stems of C1 and C4 plants were collected from three biological replicates per sample. The collected leaf and stem samples were frozen in liquid nitrogen and stored at -80°C for subsequent transcriptomic analysis.

Metabolite determination and analysis: Upon slow thawing at 4°C, appropriate amounts of samples were mixed with a pre-cooled methanol/acetonitrile/water solution (2:2:1, v/v) to ensure thorough metabolites extraction. The mixture was vortexed vigorously to homogenized then subjected to low-temperature ultrasound for 30 min to enhance the release of the metabolites. Following this, the samples were incubated at -20°C for 10 min to precipitate protein and other macromolecules. The mixture was then subjectd to and centrifugation at 14,000 g for 20 min at 4°C for the separation of the supernatant consisted of the metabolites. The supernatant was collected carefully and vacuum dried in order to remove the solvents (Römisch-Margl *et al*., 2012). For mass spectrometry analysis, the dried samples were reconstituted in 100 µL of acetonitrile/water solution (acetonitrile: water = 1:1, v/v), vortexed for complete dissolution, and centrifuged again at 14,000 × g for 15 min at 4°C to remove any particulate matter if present. The clarified supernatant was then analyzed using an Agilent 1290 Infinity LC Ultra-High-Performance Liquid Chromatography (UHPLC) system equipped with a HILIC column to separate the metabolites in the samples. An AB Triple TOF 6600 mass spectrometer was used to collect both first- and second-level spectra of the samples providing detailed metabolite profile (Chen *et al*., 2022). The original data in Wiff format were converted to mzXML format using ProteoWizard, and the XCMS software was utilized for peak alignment, retention time correction, and peak area extraction. The extracted data from the XCMS underwent metabolite structure identification and data preprocessing. Subsequently, experimental data quality evaluation and data analysis were performed (Zhang *et al*., 2022).

Transcriptome sequencing analysis: The samples were sent to Beijing Allwegene Tech Co., Ltd., where RNA extraction was performed using the RNAprep Pure Plant Kit (Tiangen, China). Qualified RNA samples were subjected to library preparation and sequencing. Before sequencing, library quality was assessed. Initially, the insert fragment size of the libraries was determined using an Agilent 2100. Subsequently, the effective concentration of the libraries was precisely quantified using Q-PCR (with a library effective concentration > 4 nM) to meet high-quality library standards. After sequencing, the raw data typically contain a small amount of adapter contamination and low-quality reads, which must be filtered to obtain high-quality sequences (clean data).

Transcript abundance is the most commonly used indicator for directly measuring gene expression levels. To achieve comparability between samples with different gene lengths, experimental conditions, and sequencing data volumes, the concept of FPKM (Fragments per kilobase of exon model per million mapped reads) was introduced. FPKM represents the number of fragments per kilobase of a gene's length in one million reads. FPKM takes into account the effects of sequencing depth and gene length on read counts and is currently one of the most commonly used methods to measure gene expression levels (Mortazavi *et al*., 2008). After obtaining gene expression levels, statistical analysis was performed on the expression data to identify significantly different genes between different samples using DESeq software. A negative binomial distribution calculation model was employed with a screening criterion of padj ≤ 0.05.

For systematic analysis of gene expression and function, the Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used (http://www.kegg.jp) (Kanehisa *et al*., 2007). Furthermore, enrichment analysis was performed for the differentially expressed genes in the KEGG database to identify the metabolic pathways associated with these genes.

Integration of metabolome and transcriptome analysis: Based on the results of metabolomic and transcriptomic analyses of differentially expressed genes, the genes and differential metabolites belonging to the same metabolic pathway were mapped on KEGG pathway maps. This allowed for further investigation of the relationship between the genes and metabolites within the pathway.

Statistical analysis: The statistical analysis employed multivariate techniques like PCA and PLS-DA to visualize patterns and enhance separation between metabolite profiles of the six potato varieties, alongside ANOVA and post-hoc tests to identify significant differences in metabolite levels, all corrected for multiple testing using FDR. For transcriptomics, DESeq was used for normalization and differential expression analysis, with significant genes identified using an adjusted p-value threshold (padj ≤ 0.05). Integrative analysis involved Pearson correlation and KEGG pathway mapping to link metabolites and gene expression, elucidating the metabolic pathways and regulatory mechanisms.

RESULTS

The agrophysiological traits of six varieties: To investigate the regulation of nutritional quality factors in potatoes, we selected six varieties, named Mincaishu 1 (C1), Mincaishu 2 (C2), Mincaishu 3 (C3), Mincaishu 4 (C4), Minshu 2 (M2), and Minshu 3 (M3) (Figure 1). These varieties exhibited comprehensive desirable traits, such as vigorous above-ground growth, thick stems, and high yield. Varieties C1–C4 are colored potatoes. Among them, C1 and C3 display a deep red color in the pericarp and pulp of the tuber. The pericarp of the C2 tuber has a slight yellow color, while the pulp is purple and yellow. The C4 tuber shows a light red epidermis, yellow pericarp, and pulp with slight flaky red coloration. The tubers of M2 and M3 are typically yellow, with the epidermis of M3 being red. Additionally, there are visible differences in the nutritional quality of the tubers among the six varieties, such as vitamin C content and yield (Table 1). Therefore, further analysis of differences in the metabolome and transcriptome is useful to reveal the molecular mechanisms affecting nutritional quality, yield, and pigmentation.

Table 1. Agronomic traits of potato cultivars examined in this study.

Name	Abbreviation	Yield (kg/mu)	Dry matter content (%)	Protein content (dry basis)	Amino acid content (mg/100g)	Vitamin C content (mg/100g)	Parent
Minshu 2	M2	2800	17.78	10.60	6.74	28.00	Golden Crown× 389746.2
Minshu 3	M3	2100	18.16	10.95	-	18.92	Chieftain× Zhengshu 6
Mincaishu 1	C1	1800	20.11	11.80	8.68	21.70	Calwhite× Zhongshu 3
Mincaishu 2	C2	2000	18.02	11.10	8.37	25.92	C79×Sable
Mincaishu 3	C3	1600	20.64	11.50	8.41	18.20	Shetland Blue×Congo
Mincaishu 4	C4	2000	19.86	-	-	29.24	Adirondack Blue×Shetland Blue

The symbol ‘-’ indicates no data.

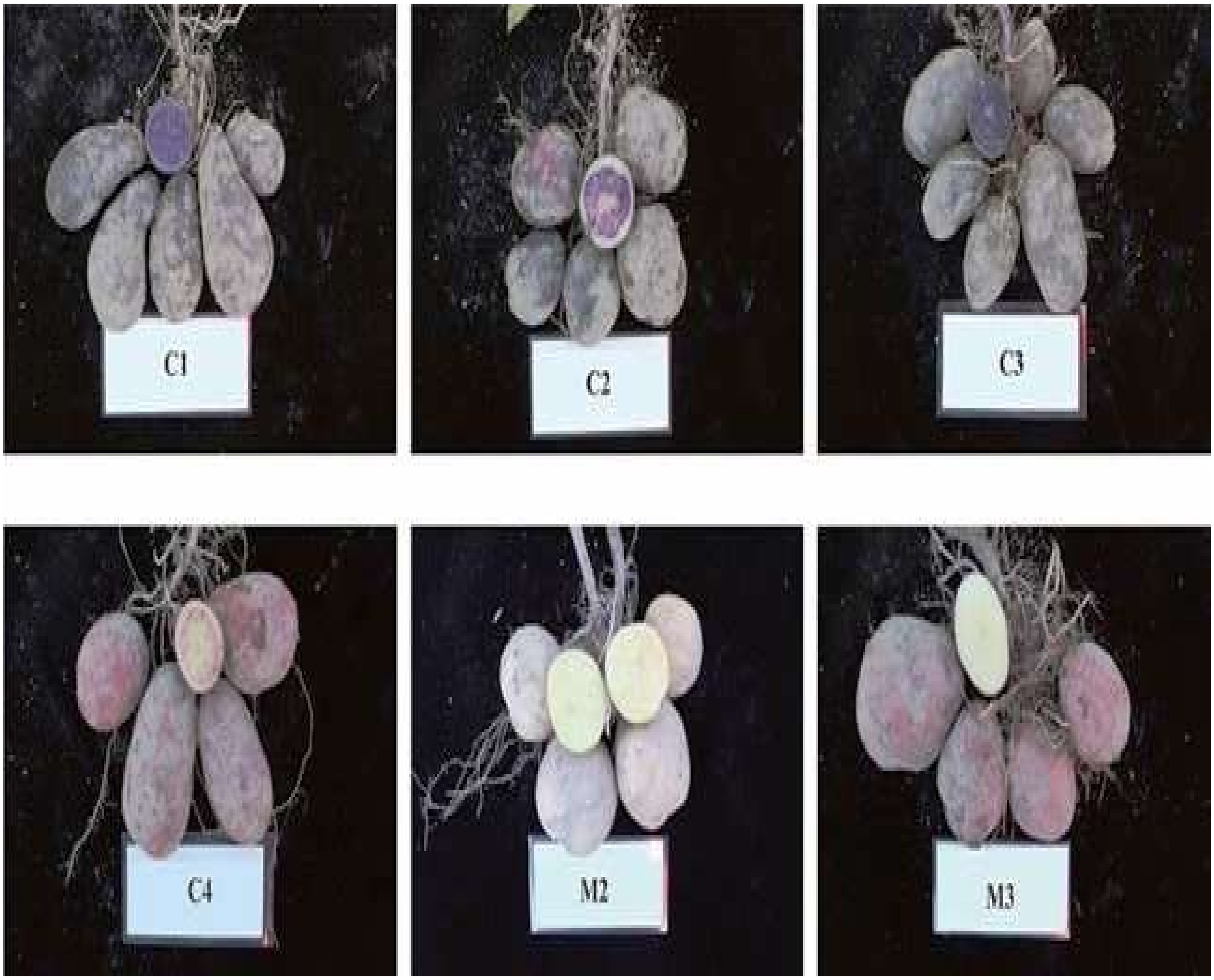

Figure 1. The mature tuber phenotypes of six varieties.

Representative photographs displaying morphological and color differences in the mature tubers among the six varieties. The photos show the interior and exterior of representative mature tubers simultaneously.

Metabolomic analysis of metabolites: To reveal the differences in metabolites among the six varieties, mature and fresh tubers were collected and subjected to metabolomic analysis. The results showed that a total of 657 substances were matched to the metabolomics reference database, primarily classified into categories such as amino acids, carbohydrates, and organic acids (Table S1). To intuitively observe the differentially expressed metabolites among the six varieties, a heat map with cluster analysis was performed. The results showed that the abundance of most metabolites in C1 was higher than in the other varieties (Figure 2). In contrast, the abundance of most metabolites in C4 was decreased compared to the other varieties (Figure 2). Notably, most of the enriched metabolites in C1 were decreased in C4, and vice versa (Figure 2). These findings indicate that the metabolism between C1 and C4 was completely reversed.

Additionally, the expression patterns of metabolites between C2 and C3, as well as M2 and M3, were similar (Figure 2). The abundance of most metabolites in these four varieties fell between C1 and C4. The Principal Component Analysis (PCA) plot also showed that C1 and C4 exhibited separation from the other varieties, except for Mincaishu 4, suggesting a high divergence existed between these two varieties. Based on these results, we found that the abundance of metabolites in C1 and C4 represented the two opposite extremes among the six varieties. Therefore, we primarily selected C1 and C4 as the target materials for further investigating the diversification mechanism.

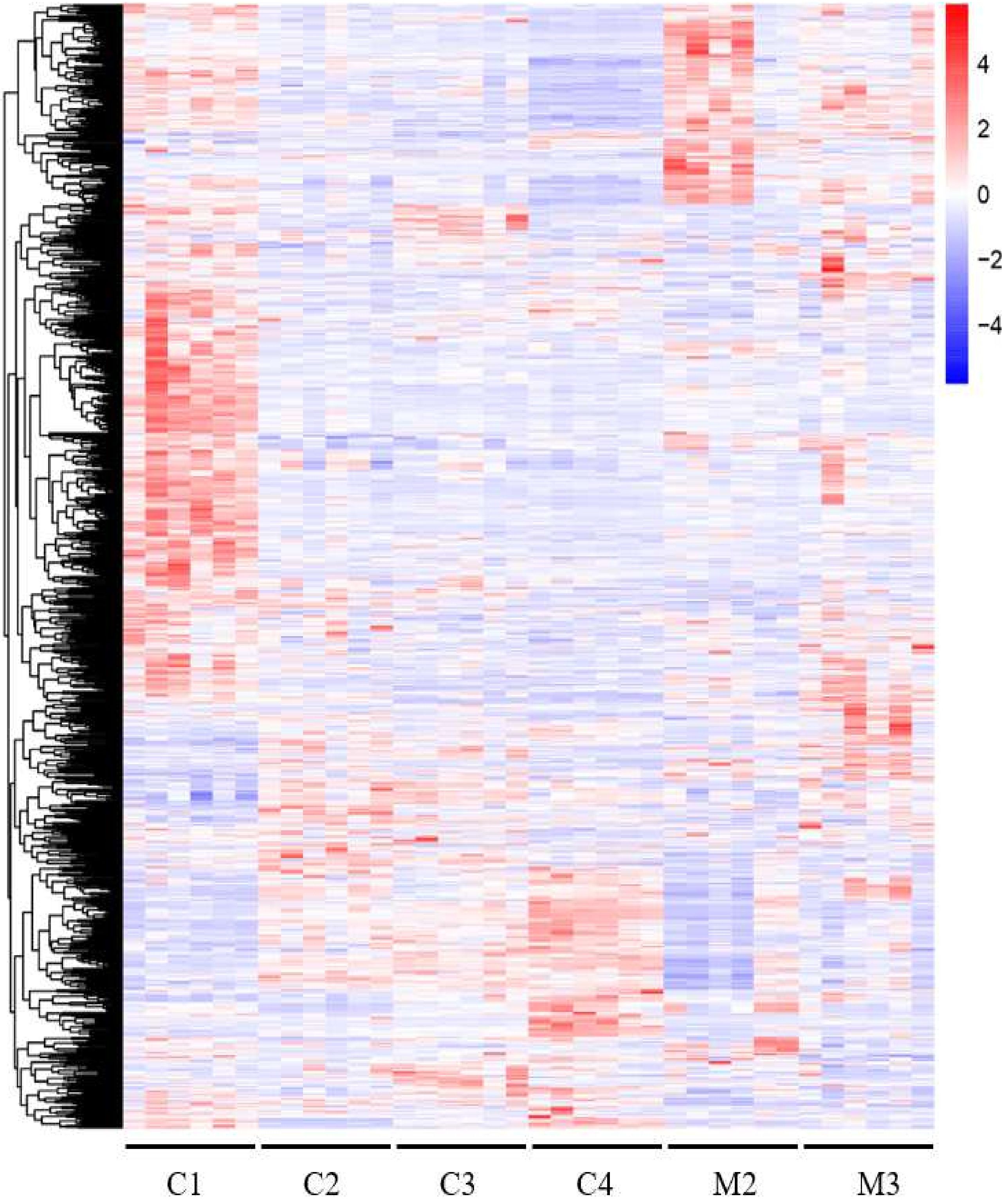

Figure 2. Cluster analysis of differentially expressed metabolites in six varieties.

Each vertical column represents metabolites that decreased (blue) or increased (red) in abundance. The horizontal column represents the same metabolite. The cluster is on the left side. Six biological replicates were used for each variety. Approximately unbiased (AU) P-values (%) are computed by 1000 bootstrap resamplings by using the R package pvclust.

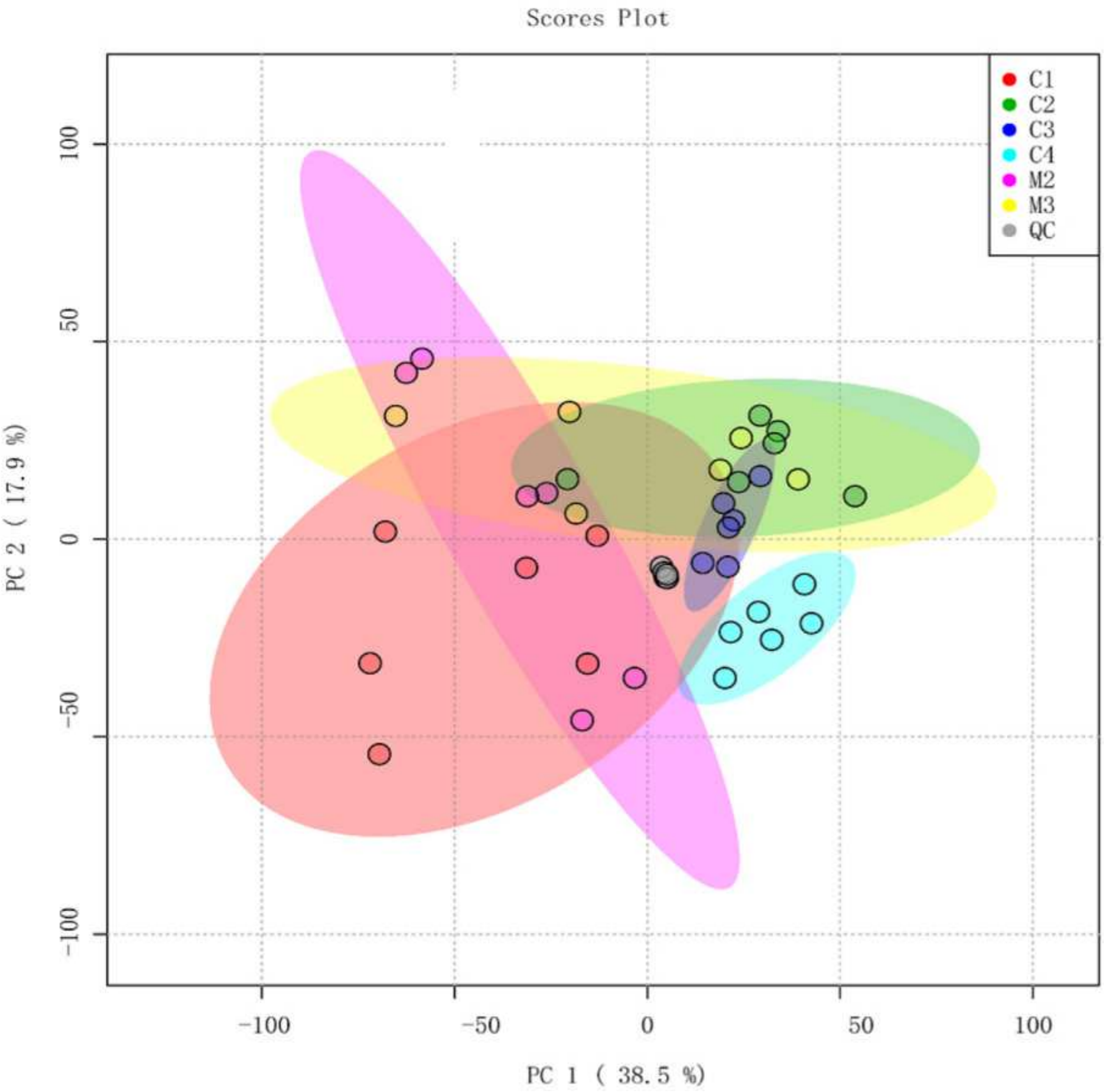

Figure 3. The Principal Component Analysis (PCA) scatter plot of six varieties.

PCA score chart based on mass spectrum data of six varieties. The six types of potato varieties were correctly classified. Six biological replicates were used for each variety. Each variety was highlighted using same-colored ellipses.

Differential metabolite analysis between C1 and C4: To analyze the differentially expressed metabolites between C1 and C4, we generated a volcano plot. The results show that 475 metabolites were down-regulated, and 190 metabolites were up-regulated in C4 compared to C1 (Figure 4A and Table S2). In other words, the 475 down-regulated metabolites in C4 were accumulated in C1 (Figure 4A). The KEGG analysis showed that the synthesis and degradation of ketone bodies, the citrate cycle (TCA cycle), as well as alanine, aspartate, and glutamate metabolism, were highly enriched in C4. Further investigation revealed that 4 metabolites, including 4-(phenylamino) phenol, S-Adenosylmethionine, and 1,2-dilinoleoyl-sn-glycero-3-phospho-(1-rac-glycerol), were significantly up-regulated in C4 compared to C1 (Figure 4B). On the contrary, 16 metabolites, such as feruloyl tyramine, guaiaverin, and 4-hydroxyatorvastatin lactone, were significantly down-regulated in C4 compared to C1 (Figure 4B). These metabolites are important molecules for normal cell function and survival (Lu, 2000; Kandel *et al.*, 2014; Sun *et al.*, 2019). In this case, these metabolites are the main components causing the difference between the two varieties.

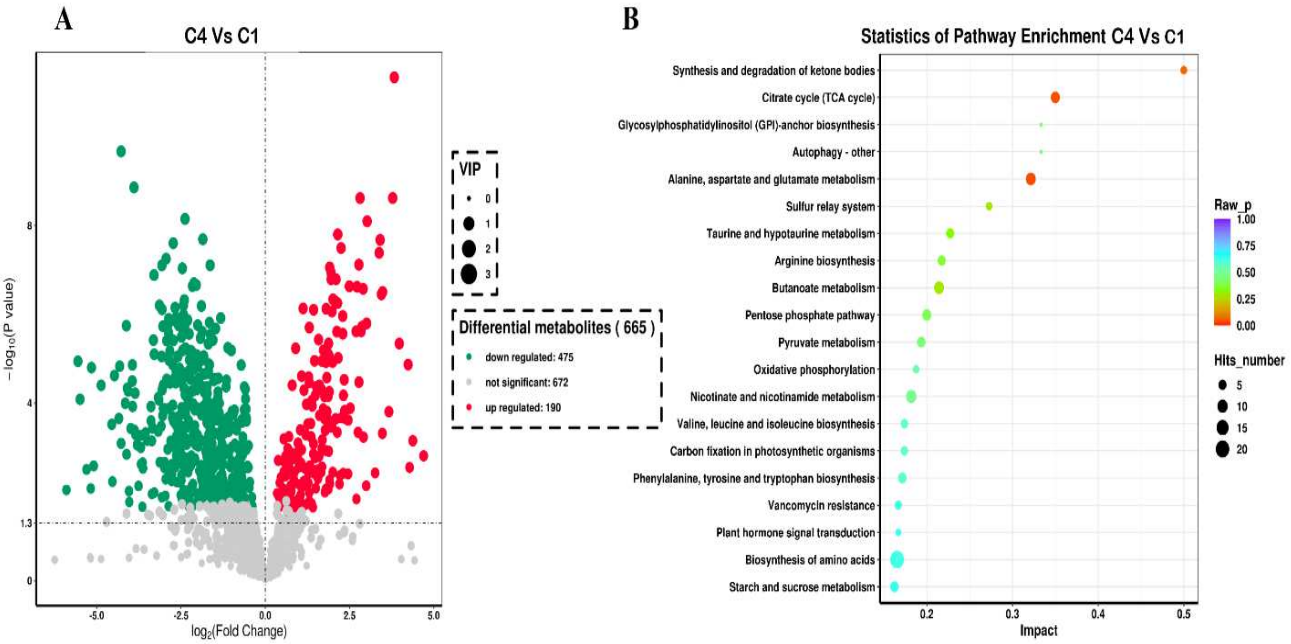

Figure 4. Analysis of differentially expressed metabolites between C1 and C4.

A, Volcano plot analysis of differentially expressed metabolites in C4 compared to C1. The blue plots indicate down-regulated metabolites, and the red plots indicate up-regulated metabolites. The gray plots indicate no significant difference. **B,** Enrichment analysis of differential metabolites in C4 compared to C1 using the KEGG database.

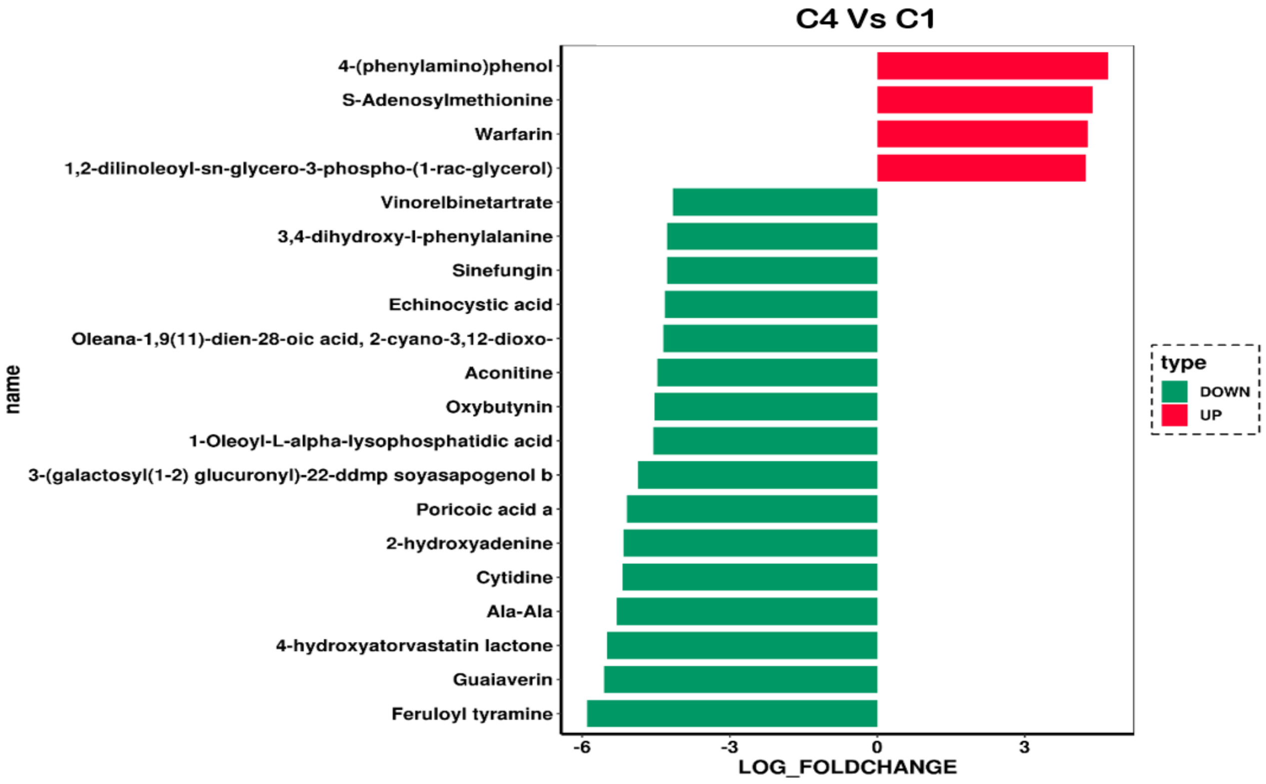

Figure 5. The significantly down- or up-regulated metabolites in C4 compared to C1.
The down- (green) and up-regulated (red) metabolites are treating as independent categories.

Transcriptome analysis of C1 and C4 varieties: To investigate the regulation of genes in C1 and C4, we performed transcriptome analysis. Total RNAs from the leaves and stems of the two varieties were extracted, respectively. After sequencing, differential gene expression between C4 and C1 was analyzed using a volcano plot. The results showed that there were 2,264 differentially expressed genes in the C4 stem compared with the C1 stem, including 880 up-regulated and 1,384 down-regulated genes (Figure 6A). In contrast to the stem, a larger number of differentially expressed genes were detected in the leaves of C4 compared to C1 (Figure 6B). A total of 10,820 differentially expressed genes were identified between C4 leaves and C1 leaves, of which 5,104 were up-regulated, and 5,716 were down-regulated (Figure 6B). These results indicate that the differential regulation of gene expression between C4 and C1 primarily occurred in the leaves compared to the stems.

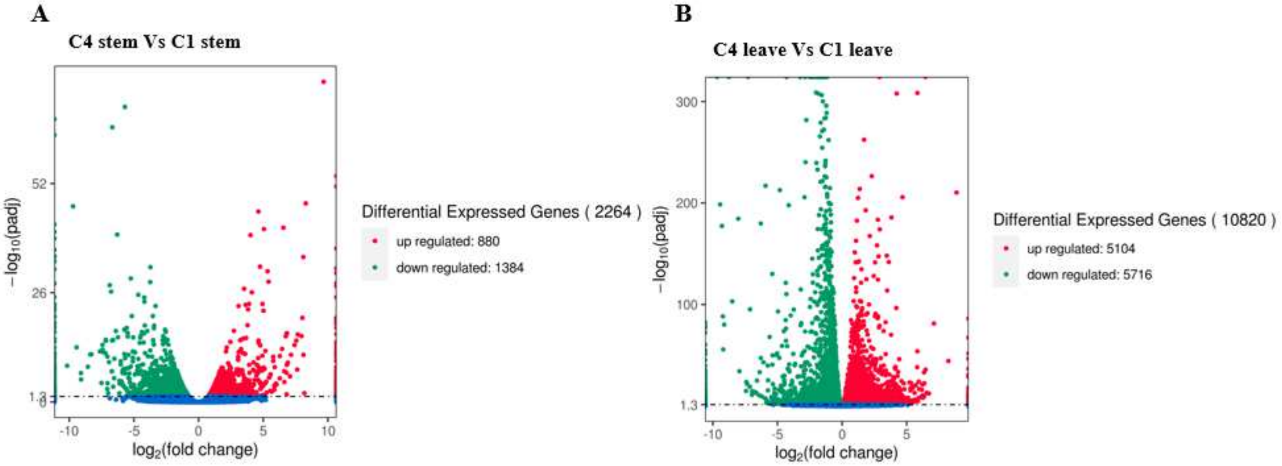

Figure 6. Volcano plot analysis of differentially expressed genes in C4 compared to C1.

A. The differential expressed genes in C4 stem compared to C1 stem. B. The differential expressed genes in C4 leave compared to C1 leave.

Transcriptome-Metabolome correlation analysis: To investigate whether the differential expression of metabolites between C4 and C1 might be related to gene expression regulation, we further analyzed the relationships between genes and metabolites within the pathways using metabolomics and transcriptomic data. Overall, 129 genes and 94 metabolites were found to be correlated in C4 and C1 (Figure 7A). Through KEGG enrichment analysis, the pathways that were commonly enriched in both the transcriptome and metabolome data were compiled and summarized. The top ten pathways with common enrichment were selected. The results showed that among the commonly enriched pathways in C4 and C1, the citrate cycle (TCA cycle) had the highest number of enriched genes and metabolites, with 41 differentially expressed genes and 7 differentially expressed metabolites (Figure 7B). This result was consistent with the significantly differential metabolites observed earlier (Figure 4B). Next was the sulfur relay system metabolism pathway with 6 differentially expressed genes and 3 differentially expressed metabolites (Figure 7B). These results imply a possibility that the citrate cycle and sulfur relay system might be the main metabolic pathways affecting the differentially expressed agro-physiological traits between C1 and C4. However, the exact metabolic pathway should be further investigated using genetic, biochemical, and molecular approaches.

Phenotypic traits are derived from the epigenetic, transcriptional, translational, and post-translational regulation of an organism's genomic information. To establish direct causal and functional associations between genotype and phenotype, it is indispensable to observe intermediate molecular levels, such as metabolite and transcript abundance. "Omics" technologies provide more holistic, multi-dimensional, multi-omics datasets that are crucial to dissect and understand the relationship between genotype and phenotype. With the development of transcriptome and metabolome technology, increasing systematic mapping of gene-metabolite associations offers new opportunities for the interpretation of more complex metabolic phenotypes (Zampieri and Sauer, 2017). For example, a recent study performed integrated metabolic and transcriptomic analysis to reveal the concentration of metabolites and gene expression for organic acids and sugars in pomegranate and further demonstrated that some genes, such as HK1, PFK7, FRK7, and INV2, were found to regulate glucose and fructose contents (Feng *et al*., 2022). In another study by Bradshaw (2019) suggested to downregulate the genes responsible for the production of tuber steroidal glycoalkaloids and acrylamide formation and has been recorded to limit the synthesis and production lower than 20 mg 100 g^{-1} fresh-weight and 750 and 500 µg kg^{-1}, respectively. This will reduce glycaemic index by increasing the amount of resistant starch; increasing protein quantity and quality; and increasing the concentrations of the minerals iron and zinc and the vitamins B9 and C. Similarly, Red-fleshed and purple-fleshed potatoes contain anthocyanins which are antioxidants and yellow-fleshed and orange-fleshed ones contain the carotenoids lutein and zeaxanthin which protect against macular eye degeneration. Genetic engineering can be used to increase dietary fiber through the introduction of inulins from globe artichoke; to increase protein quality and quantity by tuber-specific expression of a seed protein, *Amaranth Albumin* 1, from *Amaranthus hypochondriacus*; and to alter carotenoid biosynthesis to produce beta-carotene, the precursor of vitamin A (Golden Potatoes), or astaxanthin, a feed additive in aquaculture. Therefore, metabolomics can easily detect the metabolic consequences of complex traits in the regulation of gene clusters. This helps bridge the gap between genotype and phenotype.

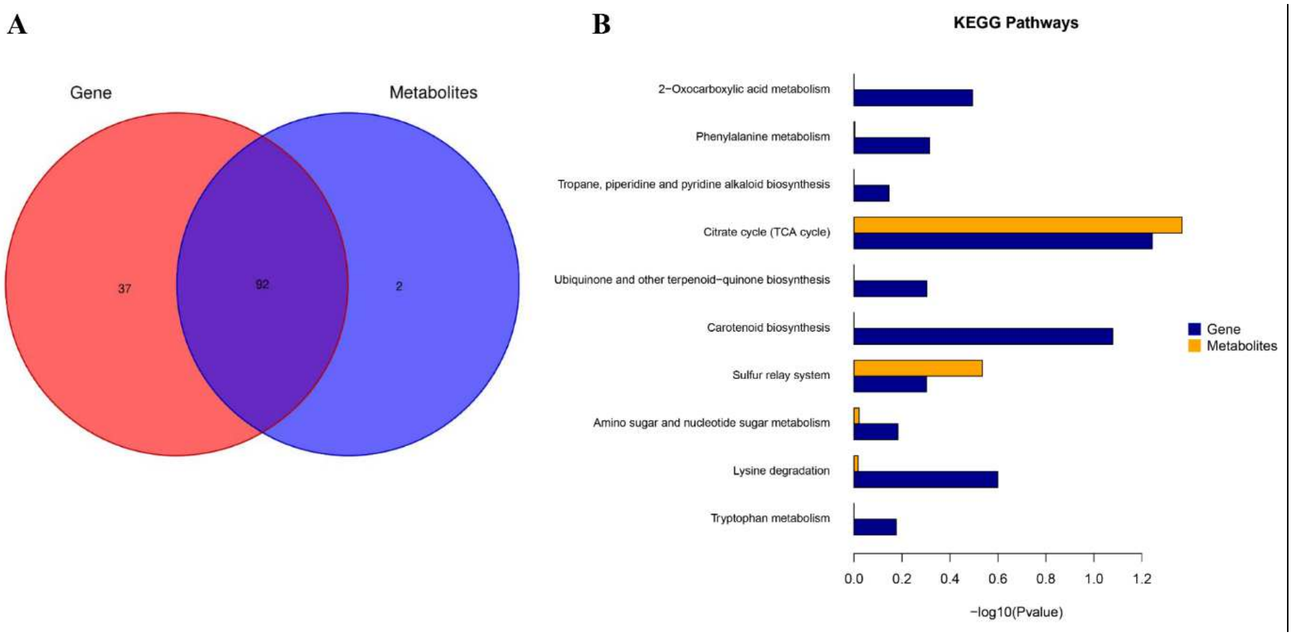

Figure 7. KEGG correlation analysis between transcriptome and metabolome in C4 and C1 varieties.

A. The correlated genes and metabolites between transcriptome and metabolome in C4 and C1 varieties. The number represent counts of correlated genes and metabolites. B. The KEGG pathway analysis of correlated genes and metabolites.

DISCUSSION

Potato, as an annual vegetable crop, has become an exceptionally high-yielding carbohydrate-rich crop due to containing a significant level of vitamin C (Brown, 2005; Alam, 2021). However, the regulatory mechanism between multiple agronomic traits is less well-known. In this case, we revealed the differences in gene expression and metabolites among different potato varieties through comprehensive transcriptomics and metabolomics analysis. By detecting 657 metabolites in the tubers, we were able to identify distinct metabolic profiles that correlate with specific genetic expression among these varieties. These differences are not merely being catalogued but have profound biological implications (Figure 2). The distribution of these metabolites was significantly different among the six varieties (Figure 3). This phenomenon was also found in the tubers of Bulgarian potato cultivars (Tomlekova *et al*., 2022). The levels of metabolites in potatoes also vary greatly, especially phenolic compounds (Andre *et al*., 2007). This implies that metabolites were highly differentiated in diverse genetic backgrounds.

Besides, we found that up to 10,820 differentially expressed genes were identified in the C4 leaves compared with the C1 leaves, of which 5,104 were up-regulated, and 5,716 were down-regulated (Figure 6B). Such a large number of differentially expressed genes between two potato varieties is rarely found. Similar but less than C4 vs. C1, a total of 8,157 genes (5,727 novel transcripts) in the flesh of the white potato cultivar 'Xin Daping' and the purple potato cultivar 'Hei Meiren' were found to be differentially expressed, with 3,685 genes up-regulated and 4,472 genes down-regulated (Liu *et al*., 2015). The variations in metabolites and gene expression reflect the inherent genetic diversity and adaptability of the potato varieties. For instance, the differential enrichment of citrate cycle-related metabolites and genes in C4, compared to C1, underscores a fundamental metabolic divergence. The citrate cycle is crucial for energy production and biosynthesis, indicating that C4 might have a higher metabolic activity and energy efficiency than C1. This could translate into better growth performance and resilience under certain environmental conditions (Marchese *et al*., 2018). Furthermore, the identification of metabolites involved in pathways such as the synthesis and degradation of ketone bodies, alanine, aspartate, and glutamate metabolism suggests that these pathways are vital for the nutritional quality and processing characteristics of the potatoes (Levaj *et al*., 2023). The enhanced understanding of these metabolic pathways provides insights into how specific potato varieties can be tailored for particular uses, whether for direct consumption, processing, or as raw materials for the food industry.

Transcriptome analysis of three different potato cultivar sprouts (light-red Hongyoung, dark-purple Jayoung, and white Atlantic) showed that only 756 and 519 transcripts had a significant change in light-red Hongyoung and dark-purple Jayoung, respectively, compared with white Atlantic (Cho *et al*., 2016). There were 482 up-regulated and 274 down-regulated transcripts in light-red Hongyoung, and 248 up-regulated and 273 down-regulated transcripts in dark-purple Jayoung, respectively (Cho *et al*., 2016). Even fewer, only 298 genes were identified as differentially expressed genes in 'Daeseo' flesh vs. 'Jayoung' flesh, while 870 genes were identified in cultured 'Jayoung' vs. 'Jayoung' flesh (Ahn *et al*., 2022). These evidences suggests that the large amount of differential gene expression between the different cultivars in potatoes C4 and C1 implies more metabolic variation in them.

Additionally, the combined analysis of transcriptomics and metabolomics in this study revealed that citrate cycle-related metabolites and genes were enriched in C4, while all of them were opposite in C1 (Figure 7B). The citrate cycle is composed of eight enzymes primarily linking the product of the oxidation of pyruvate and malate to CO_2 with the generation of NADH for oxidation by the mitochondrial respiratory chain (Fernie *et al*., 2004). Similarly, the level of transcripts of the majority of genes encoding the citrate cycle increased during the dark period in potatoes (Urbanczyk-Wochniak *et al*., 2005). Moreover, coordinated changes in the levels of these transcripts have been reported previously during development in potato tubers (Carrari *et al*., 2003). The high number of differentially expressed genes indicates that C4 and C1 have distinct regulatory mechanisms that govern their metabolic processes. This diversity is crucial as it reflects the adaptive strategies of each variety, which may be tailored to specific environmental conditions or cultivation practices (Levaj *et al*., 2023). For example, the increased expression of genes related to the citrate cycle in C4 suggests an enhanced capacity for energy production and efficient nutrient utilization, which could translate to improved growth and stress resilience (Yin *et al*., 2024). In plants, this metabolic pathway is extremely variable between species, especially the level of accumulation of the related organic acids (Nunes-Nesi *et al*., 2013). In this case, the difference in gene expression in the citrate cycle between C4 and C1 also suggests that the induction of this critical pathway of respiratory metabolism occurs during potato domestication and evolution. Understanding the metabolic and genetic differences between potato varieties provides valuable insights for breeding programs aimed at enhancing specific traits. The identification of key differentially expressed genes associated with important metabolic pathways can inform the selection of parent varieties with desirable characteristics.

Conclusion: In this study, transcriptomics and metabolomics were utilized to reveal crucial genes and metabolic pathways involved in tuber development. The findings enrich the research on tuber development pathways and support previous studies on factors affecting tuber development. Beyond advancing scientific understanding, these insights have significant practical implications for potato breeding and agriculture. By identifying key genes and metabolic pathways, this study provides valuable molecular markers that can be used in breeding programs to develop potato varieties with enhanced traits such as improved yield, stress tolerance, and nutritional quality. This research lays a solid foundation for future investigations into the mechanisms underlying tuber development and offers actionable insights for enhancing potato cultivation practices, ultimately contributing to food security and sustainable agriculture.

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Author contributions: WBL and HT designed the research; WBL performed most of the experiments; WBL, HWL, GCX, AS, YQX, RCJ, MQ, and SXQ analyzed the data. WBL and HT wrote the article.

Competing interests: The authors have no conflicts of interest to declare.

Data availability: The data that support the findings of this study are available in the supplementary material of this article.

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