

Metabolic profile and morphological characteristics of leaf tips among different sweet potato (*Ipomoea batatas* Lam.) varieties

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Abstract Sweet potato leaf tips have high nutritional values, and exploring the differences in metabolic profile of leaf tips among different sweet potato varieties can provide information to improve their qualities. In this study, a UPLC-Q-Exactive Orbitrap/MS-based untargeted metabolomics method was used to evaluate the metabolites in leaf tips of 32 sweet potato varieties. Three varieties, A01, A02, and A03, with distinct overall metabolic profiles, two varieties, A20 and A18, with distinct profiles of phenolic acids, and three varieties, A05, A12, and A16, with distinct profiles of flavonoids were identified. In addition, a total of 163 and 29 differentially expressed metabolites correlated with the color and leaf shape of sweet potato leaf tips were identified through morphological characterization. Group comparison analysis of the phenotypic traits and metabolite–phenotypic trait correlation analysis indicated that the color differences of sweet potato leaf tips were markedly associated with flavonoids. Also, the level of polyphenols was correlated with the leaf shape of sweet potato leaf tips, lobed leaf types had more higher levels of polyphenols than the entire types. The findings on the metabolic profile and differentially expressed metabolites associated with the morphology of sweet potato leaf tips would provide useful information for breeding sweet potato varieties with higher nutritional values.¹

Keywords: sweet potato, leaf tips, phenotypic traits, metabolic profile, differentially expressed metabolites, polyphenols

1. Introduction

Sweet potato (*Ipomoea batatas* Lam.) is an important food crop with high amounts of nutritional and bioactive components in different parts (tubers, leaves, stems, and stalks), and has been widely explored in recent years (Wang *et al.* 2016; Alam 2021). China is the leading producer of sweet potatoes, with an annual production of 51920944 and 49195507 tons in 2019 and 2020, respectively (accounting for 56.75 and 54.97%, respectively, of the world's sweet potato production (FAO, 2022)).

The tuberous root of the sweet potato plant is the mostly harvested organ and is widely used as food or as a raw material for starch production. The above-ground parts of sweet potatoes, including leaves, stems, and petioles, are often discarded in the fields or partly used as livestock feed (Xu *et al.*

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2010; Cui *et al.* 2011). In fact, the above-ground parts of sweet potatoes have high nutritional values. Sweet potato leaves are rich in crude protein, crude fat, crude fiber, carbohydrate, polyphenols, vitamin C, carotenoids, and minerals (Sun *et al.* 2014). In addition, sweet potato leaves have higher levels of polyphenols than the root, flesh root tissue, and sweet potato peels (Sun *et al.* 2017; Makori *et al.* 2022). Moreover, the amounts of polyphenols in sweet potato leaves are higher than in most commonly consumed commercial vegetables, such as spinach, kale, amaranth, eggplant, cabbage, cauliflower, green peas, and lettuce (Kurata *et al.* 2019; Alam 2021).

Sweet potato leaf tips include parts about 15 cm below the growth point, comprising fresh and tender leaves, stems, and petioles, which are widely used as leafy vegetables in south China (Tang *et al.* 2021; Jia *et al.* 2022). Utilization of these parts as vegetables provides humans with various nutritional compositions from sweet potato leaves, stems, and petioles. Furthermore, sweet potato leaf tips have higher production than several conventional leafy vegetables because sweet potatoes have high reproduction ability and can be harvested several times a year (Xu *et al.* 2010). In addition, sweet potatoes can be easily grown during the monsoon season in the tropics, and sweet potato leaves may be the only vegetables available in some countries after a flood or a typhoon (Islam *et al.* 2002).

Plant secondary metabolites, such as terpenes, polyphenols, and alkaloids, are key components found in edible plants and have been widely explored due to their unique physiological functions, antioxidant activity, and other health-beneficial properties (Wang *et al.* 2018). Polyphenols have a high antioxidant capacity and are associated with several significant health benefits, such as antimicrobial, antidiabetic, antiobesity, anticarcinogenic, antimutagenic, and antihypertensive effects (Al-Shabib *et al.* 2018; Makori *et al.* 2022). Previous findings revealed that polyphenols could be used for treatment of COVID-19 disease (Bahun *et al.* 2022). The stems and leaves of sweet potatoes have high levels of polyphenols, which can be utilized to improve human health. Highly nutritional sweet potato varieties can be identified by evaluating the metabolic profile of different sweet potato leaf tips and elucidating varieties with high levels of distinct metabolites (especially polyphenols).

Sweet potato was initially domesticated in Neotropical America. To date, diverse sweet potato varieties with significant morphological differences have been cultivated globally owing to human migration and distribution activities (Drapal *et al.* 2019). Leaf tips of different sweet potato varieties have unique morphological characteristics, such as diverse colors of the top buds, top leaves, mature leaves, petioles, and stems. Besides the color difference of sweet potato leaf tips, sweet potato leaf shape exhibits significant variations among different varieties (Gupta *et al.* 2020; Jackson *et al.* 2020). Therefore, exploring the specific morphological characteristic-related metabolites can provide a foundation for breeding sweet potato varieties with higher nutritional value. However, there is limited knowledge of the metabolic information for the leaf tips of different sweet potato varieties.

Metabolomics is a comprehensive metabolic profiling approach widely used for simultaneous identification and quantification of a wide range of metabolite classes in a non-biased manner (Fiehn 2002). Metabolomics is more rapid, convenient and provides high-throughput data compared with conventional analytical techniques (Koistinen *et al.* 2018; Xiao *et al.* 2022;). In this study, a Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)-based untargeted metabolomics was conducted to identify and quantify metabolites in 32 different sweet potato leaf tips. This approach was used to explore the differences in metabolic profile of leaf tips from different sweet potato varieties and unique metabolites associated with morphological characteristics. The results of the present study will provide useful information on the differences in metabolic profile among different sweet potato leaf tips and provide a basis for breeding sweet potato varieties with high nutritional

value as a source of leaf vegetables.

2. Materials and methods

2.1. Plant materials and field experiments

A total of 32 different sweet potato varieties from the National Germplasm Nursery for Sweet Potato (Guangzhou) were used as experimental plant materials (Appendix A). The sweet potato varieties were planted at the Baiyun Experimental Station (23°23'N, 113°26'E; 20 m above sea level) of Guangdong Academy of Agricultural Sciences, Guangzhou, China, on 21 July 2021, following the standard production practices. Field management conditions were maintained the same for all the varieties. Collection of phenotypic data of the sweet potato leaf tips was conducted 40-50 days after transplanting. Data of each phenotypic trait was collected following the classification and the corresponding given number as previously reported by Zhang and Fang (2006). For example, light green, green, light purple, purple and brown were used to evaluate the trait color of top bud (CTB) of all varieties (corresponding number; 1, 2, 3, 4, and 5, respectively) (Table 1).

Table 1 Evaluation phenotypic traits of the sweet potato leaf tips (Zhang and Fang 2006)

Trait	Phenotypic traits classification and the corresponding given number
Color of top bud (CTB)	1, light green; 2, green; 3, light purple; 4, purple; 5, brown.
Color of top leaf (CTL)	1, light green; 2, green; 3, purple green; 4, brown green; 5, light purple; 6, purple; 7, brown; 8, yellow; 9, red.
Color of mature leaf (CML)	1, light green; 2, green; 3, purple green; 4, brown green; 5, light purple; 6, purple; 7, brown; 8, yellow; 9, red.
Petiole pigmentation (PP)	1, light green; 2, green; 3, light purple; 4, purple; 5, dark purple.
Stem color (SC)	1, light green; 2, green; 3, mauve; 4, light purple; 5, purple; 6, dark purple; 7, brown.
Pigmentation of basic leaf vein (PBLV)	1, light green; 2, green; 3, light purple; 4, purple; 5, dark purple.
Pigmentation of basic petiole (PBP)	1, light green; 2, green; 3, light purple; 4, purple; 5, dark purple.
Leaf shape (LS)	1, entire; 3, moderate lobed; 5, deep lobed.

2.2. Sample preparation and metabolite extraction

Metabolite analysis was conducted using 15 cm long sweet potato leaf tips collected from the part below the growth point. For each variety, a sample comprised 3-5 leaf tips from individual plants was collected. The samples were collected on the same day (50 days after transplanting) and sampling was conducted within an hour to minimize differences. After collection, all samples were cut into 0.5-1 cm long sections, placed in liquid nitrogen and transferred to -80°C before extraction of metabolites.

The fresh samples were freeze-dried under vacuum conditions for at least 72 hours before extraction of metabolites. The freeze-dried samples were ground into very fine powder, then stored at -20°C for subsequent analysis.

MeOH (600 µL, stored at -20°C) (Containing 2-Amino-3-(2-chloro-phenyl)-propionic acid (4 ppm) was added to samples (50 mg) in a 2-mL centrifuge tube, then vortexed for 30 s. Glass bead (100

mg) was added to the sample, then placed in a tissue grinder at 60 Hz for 90 s. Ultrasound was conducted at room temperature for 15 min. The sample was centrifuged at 12000 rpm and 4°C for 10 min and the supernatant was obtained by filtering the sample using a 0.22- μ m membrane and transferred into a detection bottle for LC-MS analysis. Each sample had four replicates. An equal amount of each sample extract was mixed to obtain quality control (QC) samples.

2.3. LC-MS conditions

Chromatographic separation was performed on an ACQUITY UPLC system (Waters, Milford, MA, USA) equipped with an ACQUITY UPLC HSS T3 (150 mm \times 2.1 mm, 1.8 μ m, Waters) column, which was maintained at 40°C. The flow rate and injection volume were set at 0.25 mL min⁻¹ and 2 μ L, respectively. Positive ion mode and negative ion mode were used for analysis of the samples. The mobile phase and gradient used were obtained from a previous study (Fan *et al.* 2020). For positive model, the mobile phases consisted of (C) 0.1% formic acid in acetonitrile (v/v) and (D) 0.1% formic acid in water (v/v). Separation was conducted under the following gradient: 0-1 min, 2% C; 1-9 min, 2-50% C; 9-12 min, 50-98% C; 12-13.5 min, 98% C; 13.5-14 min, 98-2% C; 14-20 min, 2% C. For the negative model, the mobile phases consisted of (A) acetonitrile and (B) ammonium formate (5 mmol L⁻¹). Separation was conducted under the following gradient: 0-1 min, 2%A; 1-9 min, 2-50%A; 9-12 min, 50-98%A; 12-13.5 min, 98%A; 13.5-14 min, 98-2%A; 14-17 min, 2% A. The QC samples were run at the beginning, middle, and end of each batch.

Mass spectrometric detection of metabolites was performed on Q Exactive (Thermo Fisher Scientific, USA) with an ESI ion source and with the spray voltage of 3.8 and -2.5 kV in positive and negative modes, respectively. Simultaneous MS1 and MS/MS (Full MS-ddMS2 mode, data-dependent MS/MS) acquisition were used. Sheath gas and auxiliary gas were set at 45 and 15 arbitrary units, respectively, and the capillary temperature was 325°C. The Orbitrap analyzer scanned over a mass range of 81–1000 m/z and at a mass resolution of 70000 for a full scan. Data-dependent acquisition MS/MS experiments were performed with an HCD scan. The normalized collision energy was 30 eV and MS/MS resolving power was 17500 FWHM. Dynamic exclusion was implemented to remove some unnecessary information in MS/MS spectra.

2.4. Mass spectrum data processing

The raw data were first converted to mzXML format by MSConvert in the ProteoWizard software package (v3.0.8789) and subsequently processed using XCMS for peak identification, peak filtration, and peak alignment. The XCMS's default set were as follows: bw=2, ppm=15, peakwidth=c (5, 30), mzwid=0.015, mzdif=0.01, and method="centWave". The metabolites were identified by accuracy mass (molecular weight error <30 ppm) and MS/MS data were matched with HMDB (<http://www.hmdb.ca>), massbank (<http://www.massbank.jp/>), LipidMaps (<http://www.lipidmaps.org>), mzcloud (<https://www.mzcloud.org>), Metlin (<http://metlin.scripps.edu>), and database built by Bionovogene Co., Ltd. (BioNovoGene, Suzhou, Jiangsu, China). After normalization, only ion peaks with relative standard deviations (RSDs) less than 30% in QC samples were kept for metabolite annotation.

2.5. Statistical analysis

UPGMA dendrograms for all sweet potato varieties based on the relative content of the metabolites

were generated using the IBM SPSS Statistics 20 Software. Heatmaps, Venn plots, and upset plots were generated using TBtools version 1.098765 (Guangdong, China). Principal component analysis (PCA) was conducted using R software (www.rproject.org). Supervised partial least squares discriminant analysis (PLS-DA) of metabolites for separation of groups for each phenotypic trait was performed using MetaboAnalyst 5.0 tool (<https://www.metaboanalyst.ca/>). The VIP value from the PLS-DA model was used as the discrimination parameter for analysis of metabolites. Histograms of the numbers of metabolites were generated using GraphPad Prism 8.02 (GraphPad Software Inc., La Jolla, CA, USA). A network for metabolite–phenotypic trait correlations was constructed using the Gephi platform (based on Java Virtual Machine, version 0.9.2, <https://gephi.org/>) based on Spearman's correlation coefficients.

3. Results

3.1. Morphological differences of the leaf tips among sweet potato varieties

The 32 sweet potato varieties with large morphological variations were selected from about 2000 germplasms from the National Germplasm Nursery for Sweet potato (Guangzhou) (Appendix B). Eight phenotypic traits of leaf tips, including color of top bud (CTB), color of top leaf (CTL), color of mature leaf (CML), petiole pigmentation (PP), stem color (SC), pigmentation of basic leaf vein (PBLV), pigmentation of basic petiole (PBP), and leaf shape (LS), were used to evaluate the morphological diversity of the 32 sweet potato varieties. The results of phenotypic data are presented in Appendix C. The phenotypic traits were significantly different among the sweet potato varieties (**Fig. 1**). For instance, for CTB, 22 varieties exhibited green color, six varieties exhibited light purple color, and four varieties exhibited purple color.

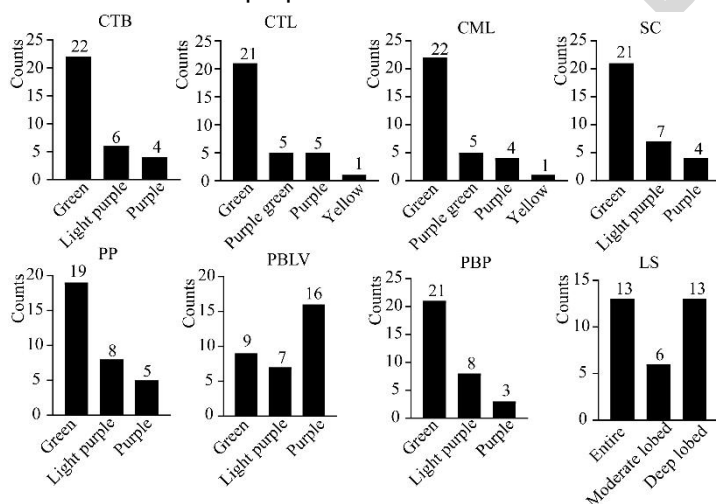


Fig. 1 The number of varieties in each group based on the eight phenotypic traits of all 32 sweet potato varieties.

PCA was used to further reveal the difference in leaf tip phenotypic traits among varieties. The distribution of varieties based on PC-1 and PC-2, showed most of the underlying phenotypic variation (73.21 %) (Appendix D). The first PC-1 explained 55.56% of the phenotypic variation, which mainly included CTB, SC, PP, and PBP; the second PC-2 explained 17.66% of the phenotypic variation, which mainly included LS and CTL (Appendix E).

3.2. UPLC-Q-Exactive Orbitrap/MS-based method is accurate for metabolite identification

A UPLC-Q-Exactive Orbitrap/MS-based untargeted metabolomic analysis was conducted to identify and quantify the metabolites in sweet potato leaf tips. The m/z (mass to charge ratio), rt (retention time), and intensity data were obtained and a total of 11368 and 8670 precursor molecules were detected under positive mode and negative mode, respectively. PCA analysis of the experimental samples and QC samples was performed to validate the results obtained from the untargeted large-scale metabolomics. The results showed that all the QC samples were clustered at the center of the PCA plot (Appendix F-A and C), indicating that the extraction and detection of samples were reliable. The ratios of characteristic peaks in QC samples with RSD <30% were 82.6 and 77.9% (Appendix F-B and D) for positive mode and negative mode, respectively, indicating that the results in the present study are reliable.

3.3. Metabolic profile analysis among sweet potato varieties

A total of 450 metabolites with good repeatability (the Pearson's correlation coefficients among the four replicates for each variety were >0.93) of the data were annotated. The detailed information, including their retention time, exact mass, molecular formula, precursor type, match percentage, CAS number, KEGG code, and relative content, are presented in Appendix G. A hierarchical cluster heatmap including 127 samples (sample A08-3 was eliminated in this study due to errors from unknown sources) based on the normalized 450 metabolites was conducted to visualize the metabolites variations among the replicates and different sweet potato varieties (Appendix H). All varieties exhibited a distinct metabolic profile, and all replicates of each variety demonstrated a clear grouping pattern. The color of the heatmap changed from red to blue, indicating a decrease in the relative content of the metabolites. The color for all replicates of each variety was relatively uniform, indicating good repeatability and reliability of the data.

The 450 metabolites were classified into 16 classes according to their basic chemical properties (Fig. 2A; Appendix I). The results showed that amino acids and derivatives comprised the largest class of metabolites, with 78 metabolites. A relatively high number of flavonoids (41) and phenolic acids (32) were also identified and quantified. The metabolites identified and quantified in this study provide comprehensive metabolic information on the edible leaf vegetable sweet potato leaf tips and has fundamental information for further study of novel metabolites in sweet potato leaf tips.

Variations of metabolites among different varieties were evaluated. The coefficients of variation (CVs) were determined for all the 450 metabolites among the 32 varieties. Significant variations were observed, with 51.11% of the metabolites having CVs above 50 and 13.33% having CVs greater than 100%. In addition, different classes of metabolites had different CVs. For instance, 53.85% of amino acid derivatives and 58.73% of organic acids had relatively low CVs (less than 50%), whereas a high number (46.34%) of flavonoids had high CVs (higher than 100%) (Fig. 2-B).

The overall metabolic profiles for all the varieties were explored and a UPGMA dendrogram was generated for all the 32 varieties based on the average relative content of the four replicates for the 450 metabolites (Fig. 2-C). Each variety had a distinct overall metabolic profile. Five main groups M1, M2, M3, M4, M5, each comprised 3, 3, 4, 2, 20 varieties, respectively, were identified based on an average Euclidean distance of 22 in the UPGMA dendrogram. The varieties A01, A02, and A03 had a distinct overall metabolic profile from the other varieties, because these three varieties clustered into M1 group and exhibited the largest distances from other groups.

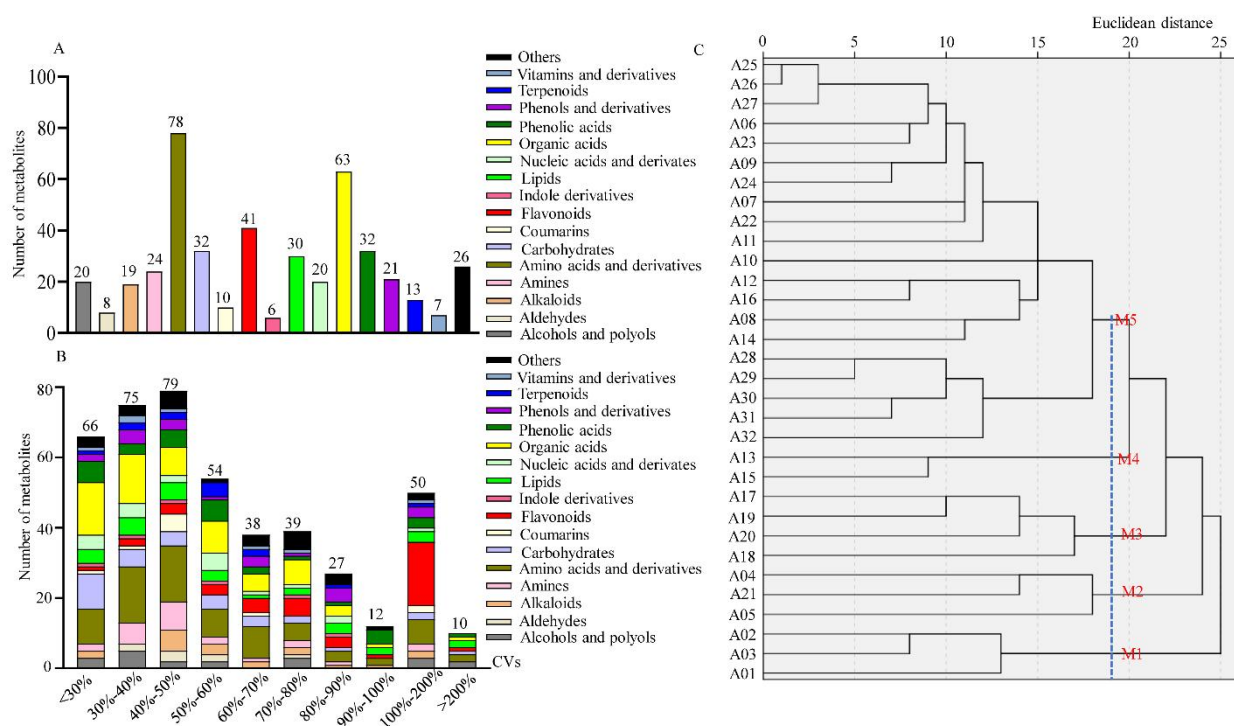


Fig. 2 Metabolic profile analysis of the 32 different sweet potato leaf tips. A, the classification of the identified and quantified 450 metabolites. B, coefficients of variation (CVs) analysis of the 450 metabolites among the 32 sweet potato varieties. C, the UPGMA dendrogram of the 32 sweet potato varieties based on the average relative content of the 450 metabolites.

3.4. Differentially expressed metabolites investigation based on the overall metabolic profile

Varieties with distinct overall metabolic profiles were grouped into five different groups according to the UPGMA dendrogram of all the identified and quantified metabolites (Fig. 2-C). The differentially expressed metabolites between varieties in different groups were further explored based on the overall metabolic profile. Supervised partial least squares discriminant analysis (PLS-DA) model was used for group pairwise comparisons analysis, and VIP value >1 in the PLS-DA model, fold change ≥ 2 or fold change ≤ 0.5 were used as threshold to determine the differentially expressed metabolites between group pairwise comparisons. Ten group pairwise comparisons were conducted, and a range of 65 to 138 differentially expressed metabolites were observed among the total 10 group comparisons (Appendix J). Venn diagrams were generated to illustrate the differentially expressed metabolites for each group compared with all the other groups (Appendix K). A total of 118, 109, 118, 116, and 126 up-regulated metabolites and 118, 124, 105, 120, and 104 down-regulated metabolites were identified for M1, M2, M3, M4, and M5 groups, respectively, compared with all other groups. A total of 72 specific metabolites, including 14 significantly up-regulated metabolites and nine markedly down-regulated metabolites were identified in group M1. Five significantly up-regulated and eight markedly down-regulated metabolites observed in group M2 (Appendix K), while, 10 significantly up-regulated and eight down-regulated metabolites were identified for group M3 (Appendix K). Furthermore, M4 group had six markedly up-regulated and 13 down-regulated metabolites, while M5 group had 1 unique up-regulated and 1 distinctly down-regulated metabolite (Appendix K). The M1 group had the largest number of distinct differentially expressed metabolites. Hierarchical cluster analysis (HCA) of the 72 distinct metabolites was conducted to visualize their relative content in the

different varieties and presented in a heatmap (Fig. 3). The HCA groups based on the relative content of the 72 specific metabolites demonstrated the same grouping pattern as the total 450 metabolites. This finding indicates that the 72 specific metabolites contributed to the main variations of all the 450 metabolites of the 5 groups. M1 group had a higher number of amino acids and amino acid derivatives, and lipids, and M3 group had a higher number of phenolic acids compared with the other groups.

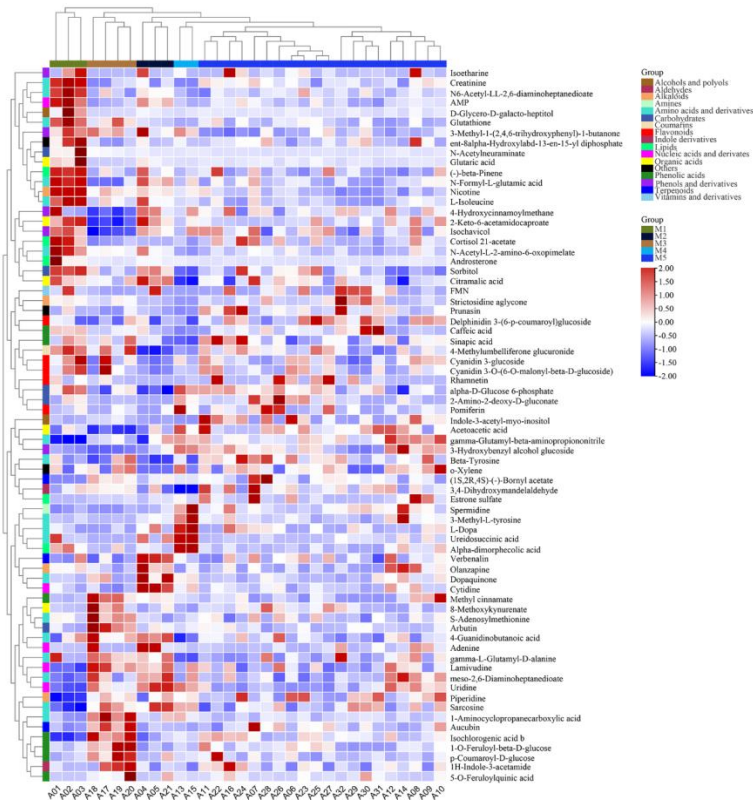


Fig. 3 Heatmap of hierarchical clustering analysis of the 72 specific metabolites of the 5 groups based on the metabolic profile. Different groups (column groups for samples and row groups for class of metabolites) were labeled with different colors (right of the heat map), the content of each metabolite was normalized to complete linkage hierarchical clustering. Each example is visualized in a single column and each metabolite is represented by a single row. Red indicates high abundance, whereas low relative metabolites are shown in green (color key scale right of the heat map).

3.5. Phenolic acids and flavonoids metabolic profile analysis among different varieties

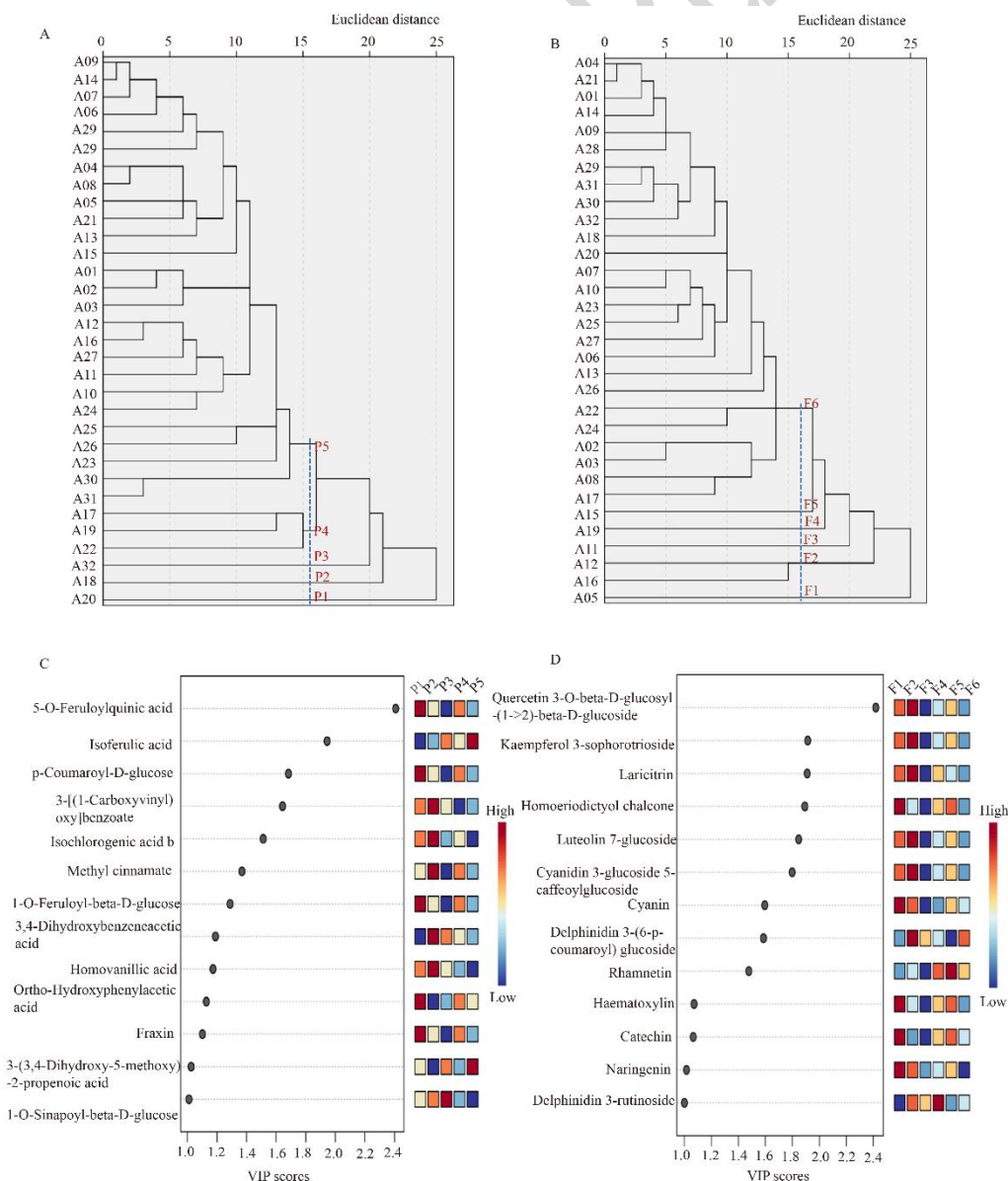
Polyphenols, especially phenolic acids and flavonoids, have remarkable benefits to humans and have been widely explored in recent years. In the present study, 32 phenolic acids and 42 flavonoids were identified from the leaf tips of the 32 sweet potato varieties. UPGMA dendrograms were generated for all the 32 varieties based on the average relative content of all the identified and quantified phenolic acids and flavonoids to explore their metabolic profile in the different sweet potato leaf tips and to identify the unique sweet potato varieties based on the profiles of unique polyphenols. Five main groups (P1 to P5) were identified based on the identified and quantified 32 phenolic acids at an average Euclidean distance of 15.5 in the UPGMA dendrogram (Fig. 4-A). The results showed that A20, A18, and A32 varieties had a distinct metabolic profile of phenolic acids. These varieties clustered in group P1, P2, and P3, respectively, and had a relatively large distance from most varieties in the other two groups (Fig. 4-A).

Six main groups (F1 to F6) were identified based on the metabolic profile of flavonoids at an average Euclidean distance of 15.5 in the UPGMA dendrogram based on the identified and quantified

41 flavonoids (Fig. 4-B). A05, A12, and A16 varieties clustered in group F1 (A05) and F2 (A12 and A16), and all exhibited purple color on most parts of the leaf tips (Fig. 1). This finding indicates that the leaf tips of the three purple sweet potato varieties had distinct flavonoid metabolic profiles from other varieties.

PLS-DA model was used to characterize sweet potato varieties with distinct phenolic acids and flavonoids based on all the separated groups. The variable importance projection (VIP) value from the PLS-DA model was used to characterize the difference of the metabolites in different groups. Metabolites with VIP >1 were selected. A total of 13 specific phenolic acids with VIP >1 exhibited significant differences among the five groups (Fig. 4-C) based on the metabolic profile of the 32 phenolic acids. The top five phenolic acids with the highest quantities in A20 of group P1 were 5-O-Feruloylquinic acid, p-Coumaroyl-D-glucose, 1-O-Feruloyl-beta-D-glucose, Ortho-Hydroxyphenylacetic acid, and Fraxin. The top five phenolic acids with the highest quantities in variety A18 of group P2 were 3-[(1-Carboxyvinyl) oxy]benzoate, isochlorogenic acid b, methyl cinnamate, 3,4-dihydroxybenzeneacetic acid, and homovanillic acid. The unique phenolic acids with the lowest quantities in variety A32 were 5-O-feruloylquinic acid, p-coumaroyl-D-glucose, methyl cinnamate, 1-O-feruloyl-beta-D-glucose, and fraxin (Fig. 4-C).

A total of 13 distinct flavonoids with VIP >1 exhibited significant differences among the six groups based on the metabolic profile of the 41 flavonoids. The three purple leaf tips varieties, A05, A12, and A16, exhibited more higher levels of the distinct flavonoids than the other varieties (Fig. 4-D). A05 variety had the top five highest levels of distinct flavonoids (homoeriodictyol chalcone, cyanin, haematoxylin, catechin, and naringenin). A12 and A16 varieties had the top six highest levels of



unique flavonoids (quercetin 3-O-beta-D-glucosyl-(1->2)-beta-D-glucoside, kaempferol 3-sophorotrioside, laricitrin, luteolin 7-glucoside, cyanidin 3-glucoside 5-caffeoylglucoside, and delphinidin 3-(6-p-coumaroyl) glucoside). The varieties with unique flavonoids and phenolic acids can be explored to provide critical information for breeding sweet potato varieties with high nutritional values.

Fig. 4 Specific flavonoids and phenolic acids analysis for varieties based on flavonoids and phenolic acids metabolic profile. A and B, UPGMA dendrograms for varieties based on relative abundance of the identified and quantified 32 phenolic acids and 41 flavonoids, respectively. C and D, Partial least squares-discriminate analysis (PLS-DA) score plot of the groups based on the dendrogram of the 32 phenolic acids and 41 flavonoids, respectively.

3.6. Differentially expressed metabolites analysis associated with the phenotypic traits of sweet potato leaf tips

Leaf tips of different sweet potato varieties exhibit significant morphological diversity, some metabolites may be associated with different phenotypic traits. In this study, differentially expressed metabolites between groups based on each phenotypic trait were evaluated to explore the different phenotypic traits related to these metabolites. Supervised partial least squares discriminant analysis (PLS-DA) model was conducted to differentiate the groups of each phenotypic trait. The PLS-DA model effectively separated all groups of the seven phenotypic traits associated with the color of sweet potato leaf tips based on differentially expressed metabolites, but some groups slightly overlapped (Fig. 5 A-G). Metabolites with VIP >1 in the PLS-DA model and a fold change >2 between groups were used to determine the differentially expressed metabolites correlated with the phenotypic traits. The results showed that 52, 74, 73, 48, 44, 28, 54, and 29 differentially expressed metabolites were correlated with CTB, CTL, CML, SC, PBP, PBLV, and PP, respectively (Appendix L-A-G). Analysis of all differentially expressed metabolites of the seven phenotypic traits showed that a total of 163 metabolites were correlated with the color of sweet potato leaf tips (Appendix M). Classification of these differentially expressed metabolites based on group pairwise comparisons of the seven phenotypic traits (Fig. 6) showed that the main differentially expressed metabolites associated with the color of sweet potato leaf tips were flavonoids. Heatmaps of the differentially expressed metabolites were generated for the seven phenotypic traits to explore the characteristics of the differentially expressed metabolites among the different groups (Appendix N). The differentially expressed metabolites exhibited significant variations among the different groups of the seven phenotypic traits. For all phenotypic traits, the purple group had the highest levels of flavonoids, and the results showed that the purple group of CTB, CTL, CML, SC, PBP, PBLV, and PP had 10, 8, 7, 5, 7, 7, and 9 up-regulated flavonoids, respectively. In addition, the yellow group of CML had 5 up-regulated phenolic acids. Anthocyanins, a subclass of flavonoids, are associated with red, purple, and blue color of plant tissues. In this study, seven differentially expressed anthocyanins associated with the color of leaf tips were identified. Cyanidin 3-glucoside 5-caffeoylglucoside was markedly up-regulated in the purple group of all the phenotypic traits, cyanin was up-regulated in the purple group of CTL, CTB, PBP, PP, and SC traits, and petunidin 3-glucoside was up-regulated in the purple green group of CTL, light purple group of CTB, and the purple group comprising PBLV and SC traits. These findings indicate that the differentially expressed metabolites can be used to explore the differences in color of sweet potato leaf tips, thus providing useful information for sweet potato breeding.

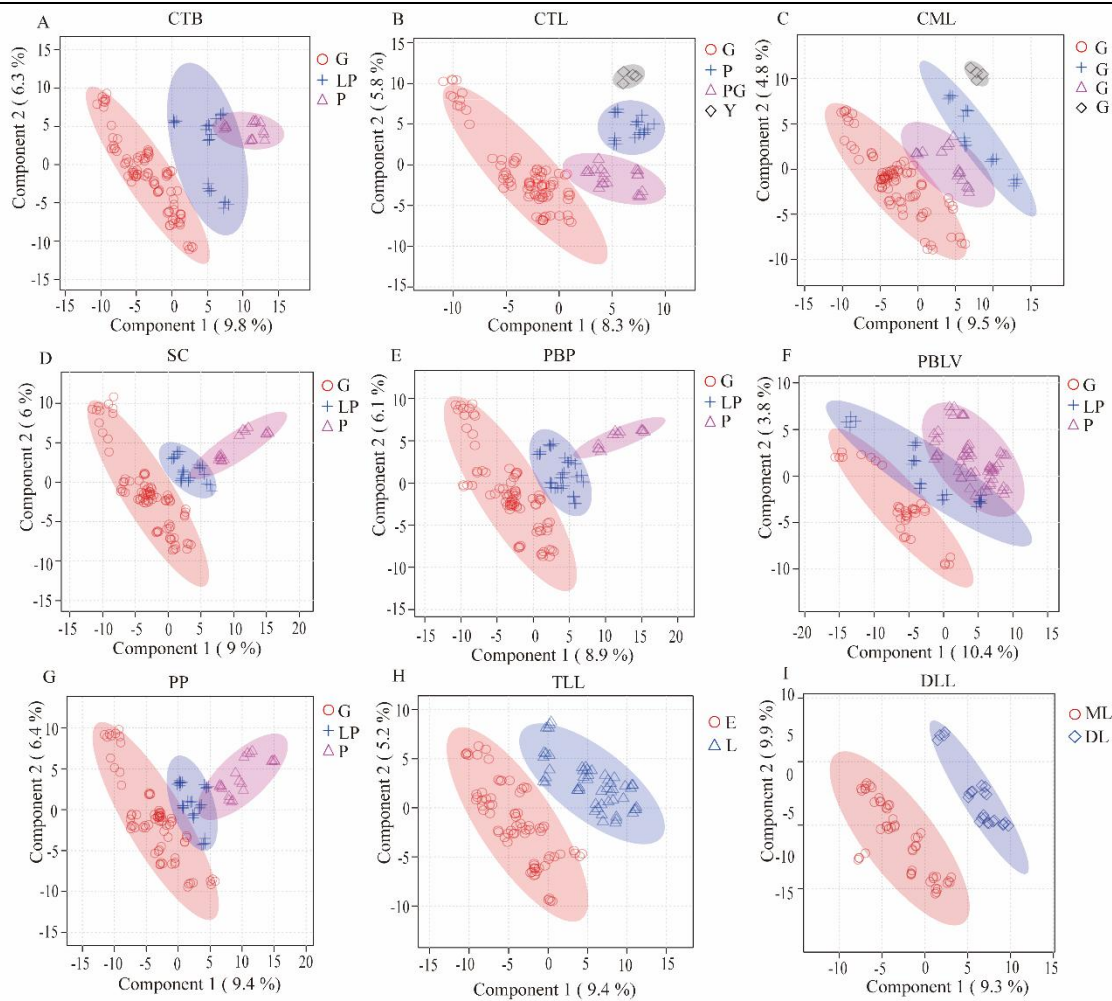


Fig. 5 PLS-DA model plots for groups separation of the eight phenotypic traits of sweet potato leaf tips based on the 450 metabolites. A-G, groups separation for the seven phenotypic traits CTB, CTL, CML, SC, PBP, PBLV, and PP, respectively. H, groups separation between the lobed and entire group of the trait LS according to the type of leaf lobes (TLL). I, groups separation between the moderate and deep lobed group of the trait LS based on the degree of leaf lobes (DLL). G, green; LP, light purple; P, purple; PG, purple green; Y, yellow; E, entire; L, lobed; DL, deep lobed; ML, moderate lobed.

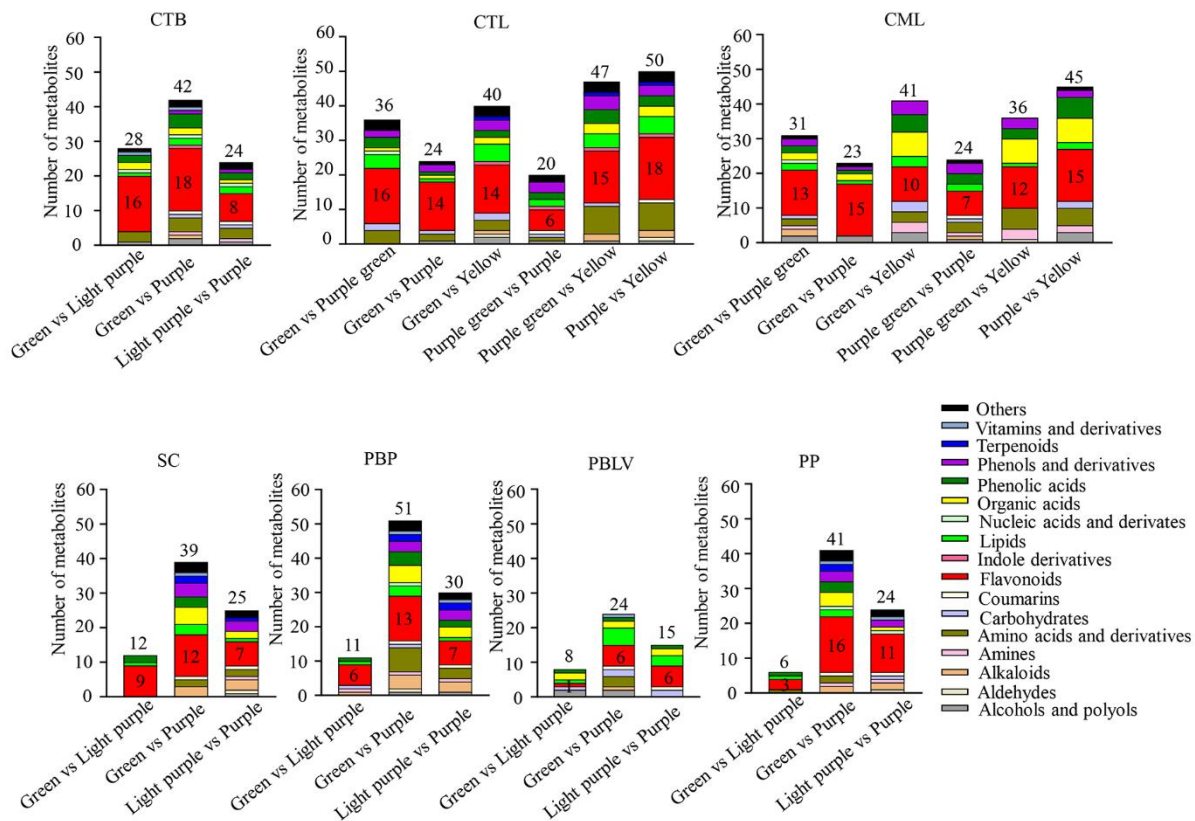


Fig. 6 Classification of the differentially expressed metabolites based on group pairwise comparisons of the seven phenotypic traits relating to the color of sweet potato leaf tips.

Sweet potato leaf shape (LS) of the leaf tips exhibited significant variations among the varieties. The 32 sweet potato varieties can be classified into two groups according to the type of leaf lobes (TLL), thirteen varieties with entire leaf shapes were in one group, and 19 varieties with lobed leaf shapes formed another group, also, the 19 varieties with lobed leaf shapes were further divided into two groups (13 deep lobed and six moderate lobed types) based on the degree of leaf lobes (DLL) (Fig. 1). Metabolite differences correlated with the leaf shape of sweet potato leaf tips were also explored. A total of 17 differentially expressed metabolites with VIP>1, fold change >2, and *P*-values<0.01, were identified between the entire and lobed leaf shape types based on the PLS-DA Model of the TLL (Fig. 5-H; Table 2). Five of these metabolites were up-regulated and 12 were down-regulated in the lobed types. The five up-regulated metabolites of the lobed types included three flavonoids (kaempferol-3-O-rutinoside, homoeriodictyol chalcone, and eriodictyol chalcone) and one phenolic acid (methyl cinnamate), which indicates that the lobed leaf types accumulate higher levels of polyphenols than the entire leaf types. Metabolites difference between the moderate and deep lobed leaf types of sweet potato leaf tips were also evaluated (Fig. 5-I). A total of 15 differentially expressed metabolites with VIP >1, FC >2, and *P*-values <0.05 were identified (Table 2) between the deep lobed and moderate lobed types. Two flavonoids (homoeriodictyol chalcone and haematoxylin) were up-regulated in the deep lobed leaf types, and three flavonoids (delphinidin 3-(6-p-coumaroyl) glucoside, rhamnetin, tribuloside) and two phenolic acids (ortho-hydroxyphenylacetic acid and 5-O-feruloylquinic acid) were down-regulated in the deep lobed leaf types. The results indicate that the polyphenols may play a vital role in discriminating different leaf shape types of sweet potato leaf tips. Furthermore, two group comparisons were conducted for lobed vs entire, and moderate lobed vs deep lobed, a total of 29 differentially expressed metabolites

were identified, and three metabolites (homoeriodictyol chalcone, L-Dopa, and S-methyl-L-methionine) showed similar differential expression patterns for the two group comparisons (Appendices L-H).

Table 2 Differentially expressed metabolites revealed relating to sweet potato leaf shapes

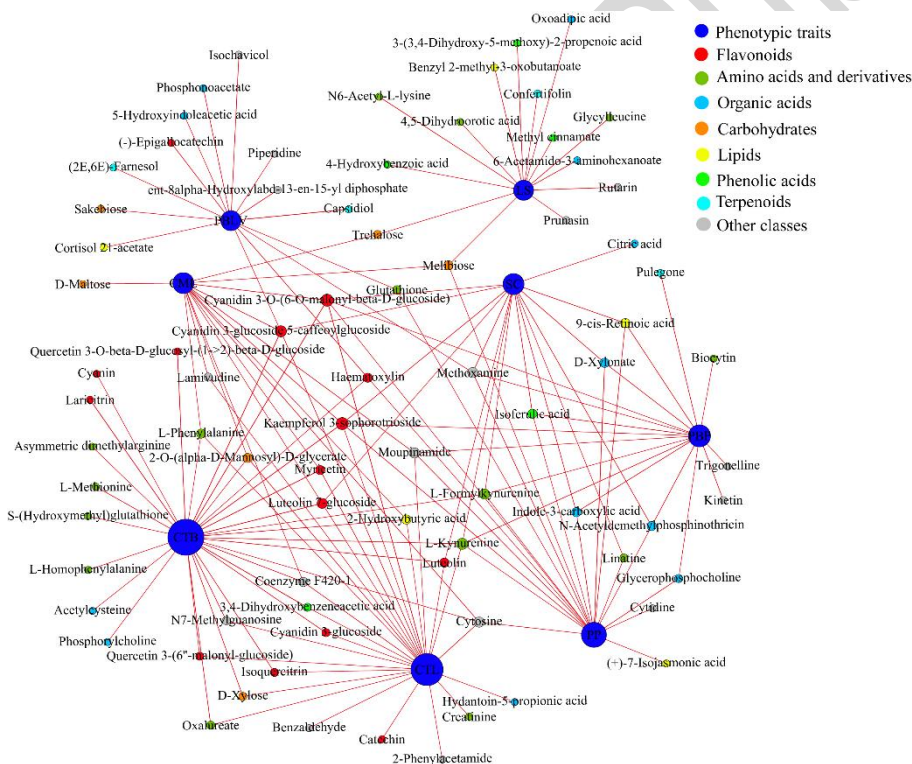
Group comparisons	Name	Class	Fold Change	VIP	<i>P</i> -value ¹⁾
Lobed VS Entire	Kaempferol-3-O-rutinoside	Flavonoids	4.578	1.485	0.0001260
	Homoeriodictyol chalcone	Flavonoids	2.365	1.225	0.0028294
	L-Dopa	Amino acids and derivatives	2.233	1.048	0.0061383
	Methyl cinnamate	Phenolic acids	2.118	2.180	0.0000000
	Eriodictyol chalcone	Flavonoids	2.102	1.820	0.0000086
	Metanephine	Phenols and derivatives	0.470	1.853	0.0000166
	S-Methyl-L-methionine	Amino acids and derivatives	0.449	1.081	0.0074706
	3,4-Dihydroxyphenylglycol	Phenols and derivatives	0.445	2.421	0.0000000
	Creatinine	Amino acids and derivatives	0.445	2.211	0.0000000
	Caffeic acid	Phenolic acids	0.423	1.695	0.0000529
	Rutarin	Coumarins	0.408	1.595	0.0002111
	FMN	Vitamins and derivatives	0.400	2.359	0.0000000
	Strictosidine aglycone	Alkaloids	0.397	1.738	0.0000064
	Sweroside	Carbohydrates	0.382	1.579	0.0005025
	Glutaric acid	Organic acids	0.351	1.554	0.0003737
	N(alpha)-gamma-L-Glutamylhistamine	Amino acids and derivatives	0.286	1.148	0.0095966
	D-Glycero-D-galacto-heptitol	Alcohols and polyols	0.270	1.874	0.0000392
Deep lobed VS Moderate lobed	Homoeriodictyol chalcone	Flavonoids	6.104	1.478	0.0018150
	Haematoxylin	Flavonoids	3.895	1.330	0.0017687
	Adenine	Nucleic acids and derivatives	3.629	1.634	0.0001410
	Estrone sulfate	Lipids	3.335	1.142	0.0147270
	Alpha-dimorphecolic acid	Lipids	2.537	1.621	0.0003512
	4-Pyridoxic acid	Organic acids	2.058	1.969	0.0010312
	L-Dopa	Amino acids and derivatives	2.053	1.692	0.0032299
	S-Methyl-L-methionine	Amino acids and derivatives	0.487	2.189	0.0000006
	1H-Indole-3-acetamide	Indole derivatives	0.472	1.399	0.0219680
	Delphinidin	Flavonoids	0.453	1.936	0.0000038
	3-(6-p-coumaroyl)glucoside	Flavonoids	0.448	1.373	0.0192420
	Rhamnetin	Flavonoids	0.448	1.373	0.0192420
	Ortho-Hydroxyphenylacetic acid	Phenolic acids	0.445	2.859	0.0000000
	Tranexamic Acid	Amino acids and derivatives	0.421	1.727	0.0044978
	Tribuloside	Flavonoids	0.291	1.561	0.0106240
	5-O-Feruloylquinic acid	Phenolic acids	0.141	2.827	0.0000016

¹⁾ *P*-value lower than 0.05 is statistically significant.

3.7. Metabolite-phenotypic trait correlation analysis

Previous studies have reported that the levels of certain metabolites are associated with morphological traits (Schauer *et al.* 2006; Hu *et al.* 2015). In this study, metabolite-phenotypic trait correlations were conducted between the eight phenotypic traits and the 450 metabolites by using Spearman's correlations. A total of 3600 metabolite-phenotypic trait Spearman's correlations were performed, among them, 149 correlations were significantly correlated (P -value <0.01).

A correlation network was established based on the 149 significantly correlated metabolite-phenotypic trait correlations, which comprised 86 nodes, including eight phenotypic traits and 78 metabolites (Fig. 7). The network also indicated that a total of 30, 26, 15, 15, 16, 14, 19, and 14 metabolites were significantly correlated with CTB, CTL, CML, SC, PBP, PBLV, PP, and LS, respectively. Analysis of the 78 metabolites significantly correlated with the phenotypic traits showed that 66 metabolites were significantly correlated with the seven phenotypic traits associated with the color of sweet potato leaf tips. Notably, flavonoids formed the highest proportion of the metabolites correlated with the seven phenotypic traits (22.7%; 15 of 66 metabolites). Six flavonoids, including cyanidin 3-O-(6-O-malonyl-beta-D-glucoside, cyanidin 3-glucoside 5-caffeoylglucoside, haematoxylin, kaempferol 3-sophorotrioside, myricetin, luteolin 7-glucoside, were significantly correlated with at least three phenotypic traits associated with the color of sweet potato leaf tips. These results imply that the color differences of sweet potato leaf tips were markedly associated with the level of flavonoids. A total of 14 metabolites were significantly correlated with the shape of sweet potato leaf tips (LS), including three phenolic acids (3-(3,4-dihydroxy-5-methoxy)-2-propenoic acid, 4-hydroxybenzoic acid, and methyl cinnamate) and one coumarin (Rutarin). This finding implies that the polyphenols may



also play a vital role in discriminating different leaf shape types in sweet potato leaf tips.

Fig. 7 Metabolite-phenotypic trait correlation network analysis of sweet potato leaf tips. The size of the dots represents the connection degree between the metabolites and the phenotypic traits.

4. Discussion

A review by Alam (2021) on health benefits of sweet potatoes indicated that in addition to the beneficial nutritional compositions, the roots and leaves of sweet potato have several constituents that promote human health and well-being, and prevent lifestyle-related diseases. Sweet potato leaf tips, a neglected important resource, can be used as leaf vegetables or as a source of diverse, healthy foods. Some distinct groups of compounds have been identified and isolated in sweet potato leaves using the traditional extraction and analysis methods (Luo *et al.* 2013, 2021; Zhao *et al.* 2014; Zhang *et al.* 2015). However, extensive evaluation of the metabolites in sweet potato leaf tips has not been conducted. Therefore, assessment of the metabolic profiles among different varieties can be conducted to explore the most nutritionally valuable varieties to improve their qualities. Metabolomics is an important technology for profiling metabolites owing to advances in liquid chromatography coupled with mass spectrometry. Untargeted metabolomics methods have been used to accurately characterize and quantify metabolites in various plants, such as lettuce (Garcia *et al.* 2016), *Acer truncatum* (Gu *et al.* 2019), and *Clausena lansium* (Fan *et al.* 2020). In the present study, a total of 450 metabolites in the leaf tips of 32 sweet potato varieties, including 41 flavonoids and 32 phenolic acids, were identified and quantified through untargeted metabolomics. Reliability and reproducibility of the data and methodology were validated by the PCAs, correlation analysis, and hierarchical cluster analysis using four replicates. Three varieties A01, A02, and A03, with distinct overall metabolic profiles, were identified based on the metabolic profile of the 450 metabolites. Varieties A20, A18, and A32 exhibited distinct and differentially expressed phenolic acids, and varieties A05, A12, and A16 exhibited distinct and differentially expressed flavonoids. The metabolomics strategy used in this study can be used to characterize the metabolic profile of other sweet potato germplasms or other plants. The method is also an effective way to identify unique varieties with differentially expressed metabolites.

Flavonoids are an important group of polyphenolic compounds with diverse biological activities in plants and benefit human health as protective dietary agents (Khalid *et al.* 2019). Flavonoids are mainly found in flowers, leaves, and seeds and are associated with the color of different plant tissues (Shen *et al.* 2022). Anthocyanins, a subclass of flavonoids, are responsible for the red, purple, and blue colors of plant tissues. Anthocyanins have several health-promoting benefits, such as protection against UV radiation, disease resistance, and protection against herbivores and pathogens (Kong *et al.* 2003; Shen *et al.* 2022; Zhao *et al.* 2022). In previous studies, Wang *et al.* (2018) indicated that the differences in flavonoid metabolic profile explained color differences in the fresh roots of five sweet potato varieties, the levels of the seven anthocyanins identified in that study were markedly high in purple-fleshed sweet potatoes than the white-fleshed sweet potatoes. Zhang *et al.* (2022) revealed that the content of flavonoids was significantly lower in the cream-fleshed mutant compared with the wild purple-fleshed sweet potato. Zhao *et al.* (2022) reported that the levels of anthocyanin metabolites are significantly lower in a sweet potato mutant with a green color for the leaf vein base and yellow color for the root skin than in the wild-type with a purple color for the leaf vein base and red color for the root skin. In the present study, differential metabolite analysis and metabolite-phenotypic trait correlation analysis indicated that the color differences of sweet potato leaf tips were significantly correlated with the content of flavonoids. Levels of the six differentially expressed flavonoids (cyanidin 3-O-(6-O-malonyl-beta-D-glucoside, cyanidin 3-glucoside 5-caffeoylglucoside, haematoxylin, kaempferol 3-sophorotrioside, myricetin, luteolin 7-glucoside) were significantly correlated with the color of sweet potato leaf tips. Varieties with purple color had higher

levels of flavonoids relative to the other varieties. The anthocyanin, cyanidin 3-glucoside 5-caffeoylglucoside exhibited the highest content in the purple group for all the phenotypic traits relating to the color of sweet potato leaf tips. These results are consistent with previous findings, which imply that the untargeted metabolomics method used in this study is reliable, and the identified differentially expressed metabolites can be used for further study to improve sweet potato leaf tips nutritional qualities.

Plant leaf shape is an important characteristic of leaf morphology. Leaf morphology significantly affects a plant's adaptation, quality, and yield (Zhu *et al.* 2016; Hu *et al.* 2018). Plants with lobed leaves have several advantages, for instance, zucchini (*Cucurbita pepo* L.) with deeply lobed leaves are suitable for high-density planting and large-scale production (Bo *et al.* 2022). However, the metabolite differences associated with plant leaf shape are yet to be elucidated. In the present study, 29 differentially expressed metabolites associated with the leaf shape of sweet potato leaf tips were identified. The findings showed that three flavonoids and one phenolic acid were distinctively up-regulated in the lobed leaf types. A previous study reported that two copies of chalcone synthase (CHS) are associated with the broadness and type of lobe of leaves in sweet potatoes and were negatively correlated with the narrow leaves with no lobes (Gupta *et al.* 2020). CHS enzyme catalyzes the first committed step in flavonoid metabolism (Chen *et al.* 2017; Khalid *et al.* 2019), which implying that the lobed leaf sweet potato leaf tips may have higher expression levels of the polyphenols (flavonoids) metabolism pathway-related genes, leading to the accumulation of higher levels of polyphenols (flavonoids). Flavonoids play essential roles in mediating plants' response to biotic and abiotic environmental factors, these compounds protect plants against biotic stressors such as herbivores, bacteria, and fungi and abiotic environmental stressors such as ultraviolet (UV) light (Shen *et al.* 2022). These results indicate that the sweet potato leaf tips with lobed leaf type may accumulate higher levels of polyphenols (flavonoids), thus, enhancing resistance to biotic and abiotic stresses and improve environmental adaption.

All sweet potato leaf tips are edible, revealing that the metabolic profile and the morphological characteristics-related metabolites in different sweet potato leaf tips can be used to identify the most nutritional varieties used as leafy vegetables. However, different sweet potato leaf tips have significantly different taste, not all varieties of sweet potato leaf tips are suitable for leafy vegetables. Therefore, discovering the specific metabolites related to the taste of sweet potato leaf tips is crucial for breeding the most suitable varieties for vegetable use. In this study, six (A04, A09, A11, A16, A27, A30) of the 32 sweet potato varieties have good taste and are suitable for vegetable use (vegetable use group), while the other 26 varieties are not suitable for vegetable use (non-vegetable use group). The non-vegetable use group varieties are mainly harvested for their roots. In this study, the PLS-DA model was also conducted for the vegetable use and non-vegetable use groups (Appendix O), and eight differentially expressed metabolites (VIP >1, FC >2, and *P*-values <0.05) were identified between the two groups (Appendix P), of which one phenol and its derivative (Isoetharine) was up-regulated in the vegetable use group. Moreover, seven of the eight metabolites, including two carbohydrates (2-amino-2-deoxy-D-gluconate, and sakebiose), an amine ((1H-indol-3-yl)-N-methylmethanamine), an organic acid (mandelic acid), an alcohol and polyol (14-dihydroxycornestine), a flavonoid (tribuloside) and a lip (aflatoxin G2) were down regulated in the vegetable use group. These differential metabolites may be associated with the taste of sweet potato leaf tips. However, the taste of sweet potato leaf tip is a very complex trait, and thus the real specific metabolites affecting the taste of sweet potato leaf tips require further verification.

Different sweet potato varieties have significant diversity in chemical compositions, each variety has its unique nutritional properties. Therefore, it is important to identify the most nutritional varieties

with highest contents of specific beneficial metabolites. Moreover, further studies should be conducted to explore the genetic locus of the specific beneficial metabolites and to discover the specific metabolites relating to the taste of sweet potato leaf tips.

5. Conclusion

To the best of our knowledge, this is the first study to evaluate the metabolic profile and differences among different sweet potato leaf tips and explore the morphological characteristics related to the differentially expressed metabolites. In this study, 450 metabolites were identified and quantified, the results showed significant variations in the expression levels of these metabolites among the sweet potato varieties. Three varieties, A01, A02, and A03, exhibited a distinct overall metabolic profile. Moreover, three varieties, A20, A18, and A32, showed a different profile of phenolic acids, while the other three varieties, A05, A12, and A16, exhibited a distinct flavonoids metabolic profile. A total of 163 differentially expressed metabolites associated with the color of leaf tips were identified in this study. Differential metabolite analysis and metabolite-phenotypic trait correlations analysis indicated that flavonoids were responsible for the significant variations in color differences of sweet potato leaf tips. Also, the polyphenols were found correlated with the leaf shape of sweet potato leaf tips, the varieties with lobed leaves had higher levels of polyphenols. These results provide fundamental information for understanding the differences in metabolites among different sweet potato leaf tips. The findings provide a basis for further metabolomic studies to potentially improve the quality of sweet potato leaf tips, enhance stress resistance and nutritional properties.

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Conflict of interest

The authors declare that they have no conflict of interest.

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