Metabolite Profiling of Different Sweet sorghum cultivars Seeds

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Research article

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Abstract

Background Sorghum bicolor is one of the most important cereal crops in the world and is widely grown in arid or semi-arid areas. Results This study focused on the metabolic Diversity of three Sweet sorghum cultivars with white, red, and purple seeds to elucidate the factors responsible for the differences in Seed color. We found 651 metabolites were divided into 24 categories including Lipids_Fatty acids, Lipids_Glycerolipids, Flavonoid, Benzoic acid derivatives, Anthocyanins, Nucleotide and its derivates, etc. Through clustering analysis, principal component analysis (PCA), and orthogonal signal correction and partial least squares-discriminant analysis (OPLS-DA), different samples were clearly separated. It shows that contained metabolites of three groups are quite different. There are 217 significantly different metabolites between Z27 and HC4 (106 down-regulated, 111up-regulated), 240 between Z6 and HC4 (58 down-regulated, 182 up-regulated), 199 between Z6 and Z27 (54 down-regulated, 135 up-regulated). Venn diagram analysis indicated that 45 the differential metabolites were common to all three comparison groups. Conclusions This study provides new insights into the differences of metabolites between different color seeds and provides theoretical basis for the sufficient utilization of Sweet sorghum cultivars.

Background

Sweet Sorghum (Sorghum bicolor) is one of all-important cereal crop in the world. It has not any remarkable stress tolerance, but also possesses high photosynthetic efficiency. It is widely planted in arid or semi-arid areas. Sweet sorghum, just like common grain sorghum, It grows widely in all regions with various landraces[1]. It can grow in harsh environments while achieving high yields As we all know, the main cause of plant color change is the difference in metabolites which contains primary metabolites and secondary metabolites [2, 3]. Primary metabolites are necessary for life-sustaining activities and growth. Although their primary metabolites are high in content, it has very few varieties. Secondary metabolites are more involved in plant disease resistance, stress resistance and other environmental responses [4]. For example, the red color of strawberry is determined by anthocyan in the purple and color of blueberry is determined by flavonoids. The red color of tomato is determined by carotenoid, and the nitrogenous substance, such as alkaloid, has anti-tumor function, mainly due to biological Alkali substance. Flavonoids that involve flavones, flavonols, anthocyanins, flavanones, and Chalcones is a class of important plant secondary metabolites [5]. Many of colored fruits and flowers Contains a large amount of Flavonoids, which is conducive to protection against damage by dormancy, ultraviolet light and phytopathogens, and free from biotic and abiotic stresses[6]. research indicated that it is very helpful to some patients with chronic diseases which include cardiovascular disease, certain types of cancer[7], and inflammatory diseases. due to their antioxidant activities[8], recently, anthocyanins is a subgroup of flavonoids, has been paid more and more attention.Many plants, just like fruits, vegetables, and flowers, contains more than 600 anthocyanins which have been identified [9-11]. Purple and dark colors depend on the anthocyanin derivatives of delphinidin, petunidin, and malvidin. The derivatives of cyanidin and pelargonidin are sources of bright-red-colored fruits[12].

In order to better understand the mechanism of change of these metabolites, Metabolomics is increasingly favored by researchers. The main purpose of metabolomics analysis is to detect and screen biologically important Metabolites from biological samples [13]. Based on this, we hope clarify the metabolic processes and mechanisms of changes in organisms. Metabolomics analysis includes two major parts of metabolomics experiments and data analysis which mainly includes metabolite screening and metabolic pathway analysis. Based on experimental design, sample collection and processing, we can identify the metabolites and the quality control of the sample data. Some different metabolites were screened out so as to perform functional prediction on the metabolites.

Methods

Planting process

The three sweet sorghum cultivars have excellent quality characteristics and are local cultivars of Tongliao in Inner Mongolia. The color is divided into white, namely Z6, red namely Z27and black, namely HC4. Z6 is a homozygous material after 6 generations of self-fertilization, female parent Z591, male parent HT6. Z27 is a homozygous material after 6 generations of self-interest, female parent ZH1, male parent T27. HC4 is a homozygous material after 6 generations of self-fertilization, female parent H9, male parent C4. The seeds used in the experiment were provided by the Sorghum from Institute of the Tongliao Academy of Agricultural Sciences, however, voucher specimens of all materials were not deposited in a publicly available herbarium. The experiment was conducted at the Experimental Base of the Agricultural College of Inner Mongolia University for Nationalities, Tongliao City, Inner Mongolia Autonomous Region. The geographical position is between 42°15'-45°41' north latitude and 119°15'-123°43' east longitude. The number of days with winds above 8 can reach 20 to 30 days. The soil in the experimental field is gray meadow sand. The soil organic matter is 26g/kg and the alkali nitrogen is 62mg/kg. The quick-acting phosphorus is 38mg/kg, quick-acting potassium is 184mg/kg and the pH value is 8.3. The trial used a randomized block design with 3 replicates in Planting 16 rows per plot, with a length of 5m, a row spacing of 0.25m, a plot area of 20m2, and a spacing of 0.5m. Seeding is carried out by means of aerial seeding. The compound fertilizer was applied at the time of sowing with 750 kg/hm2, and the others were consistent with the production management of farmland. All sweet sorghum cultivars were sown on April 28, 2018. The bag is put at the flowering stage, and Mesh bag is alternative at the end of the flowering period. After ripening, three sweet sorghum seeds are harvested from each plant.

Sample preparation and extraction

Three different colored seeds were obtained base on color, which was divided in three goup, Z6 group in white, Z27 in red, HC4 in black and three replicates in every group. Every replicate, all of the sample, amixture of seeds from 5-6 plants for metabolomics, was washed and stored in -80 °C for further analysis.

We used a mixer mill (MM 400, Retsch), a zirconia bead for 1.5 min at 30 Hz, to crush the freeze-dried seed. Aqueous methanol (1.0 ml 70%) was employed to extract sample of 100mg powder overnight at 4℃. After Centrifuge in 10 minutes for sample, the extracts were absorbed (CNWBOND Carbon-GCB SPE Cartridge, 250mg, 3ml; ANPEL, Shanghai, China) and filtrated (SCAA-104, 0.22μm pore size; ANPEL, Shanghai, China). Subsequently, we analyzed the sample extracts by using an LC-ESI-MS/MS system (HPLC, Shim-pack UFLC SHIMADZU CBM30A system; MS, Applied Biosystems 6500 Q TRAP).

Qualitative and quantitative principles of metabolites and data analysis

We obtained Linear Ion Trap (LIT) and triple quadrupole (QQQ) scans on a triple quadrupole-linear ion trap mass spectrometer (Q TRAP), API 6500 Q TRAP LC/MS/MS System, equipped with an ESI Turbo Ion-Spray interface, operating in a positive ion mode and controlled by Analyst 1.6.3 software (AB Sciex).The collision gas (CAD) was high. Instrument tuning and mass calibration were performeded in 10 and 100 μmol/L polypropylene glycol

solutions in QQQ and LIT modes, respectively. QQQ scans were acquired as MRM experiments with collision gas (nitrogen) set to 5 psi. DP and CE for individual MRM transitions were done with further DP and CE optimization. A specific set of MRM transitions were monitored for each period according to the metabolites eluted within this period. The monitoring mode was set to multiple-reaction monitoring (MRM) quantified the Metabolite. Principle component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) were performed to analyze metabolite cultivars-specific accumulation based on the described[14].

The pheatmap package, in R software (version 3.5.1), was used to draw Heatmap plots [15]. Before it, Log 2 transformation of metabolite relative response values was conducted. R and SPSS19 software (IBM Corp., Armonk, NY, USA) were performed to calculate the area under the receiver operating characteristics (AUC) values, sensitivity (SE) and specificity (SP) of the potential biomarkers to evaluate the differential performance of metabolites[16].

The Kyoto Encyclopaedia of Genes and Genomes (KEGG) Pathway database (http://www.kegg.jp/kegg/pathway.html) is centered on metabolic reactions and make a connection metabolic pathways and corresponding proteins[17]. The enrichment analysis and topological analysis of the pathways where differential metabolites are present enables further screening to identify the key pathways that show the highest correlation with metabolite differences[18].

Results

Agronomic trait of three sweet sorghum cultivars

As shown in the Table1, after planting on May 22, compared with the other two groups, the time from emergence to heading, flowering, and the time from emergence to flowering days and the entire growth period is much longer in the Z6 group. Spike stalk protruding state, Ear shape and Seed color are different in three groups. Especially seed color change Very large (Figure 1), with Z6 possessing white, Z27 and Hc4 possessing red/black. In order to explore the mechanism of color change, we then performed a metabolome analysis.

Principal component analysis (PCA) and OPLS-DA for three groups

Principal component analyses of samples (including quality control samples) provide an initial understanding of the overall metabolic differences between groups and the degree of variability between samples within the group. The metabolite profiles of these three group were plotted onto a PCA[18]. The two principal components, PC1, PC2 separately account for 39.06%, 26.2% (Figure2A). The PCA analysis showed that three groups were distinctly separated, and the repeated samples were compactly gathered together, thus indicating that the experiment was reproducible and reliable (Figure2A).OPLS-DA that maximize the distinction in different groups was used to find differential metabolites. Q 2, a best parameter to evaluate the model in OPLS-DA, above 0.9 indicated an excellent model. In this study, OPLS-DA model showed that all comparison groups exceeded 0.9 between Z6 and Z27 (R 2 Y =1, Q 2 = 0.964; Figure2B), between Z6 and HC4 (R 2 Y = 1, Q 2 = 0.969; Figure2C), between Z27and HC4 (R 2 Y=1, Q2=0.973; Figure2D). It proved that these models were reliable and could be conducted to screen for differential metabolites.

Metabolic Profile during three groups

Based on the results of OPLS-DA, from the obtained multivariate analysis of the variable importance in project (VIP) of the OPLS-DA model, metabolites with different varieties or differences between tissues can be initially screened. At the same time, the differential metabolites can be further screened by combining the p-value or the fold change of the univariate analysis. The criteria for screening: the fold change value of ≥ 2 or ≤ 0.5 and the VIP value of ≥ 1. A total of 366 differential metabolites were found meanwhile, 217 significantly different metabolites between Z27and HC4 (106 down-regulated, 111 up-regulated), 240 between Z6 and HC4 (58 down-regulated, 182up-regulated), 199 between Z6 and Z27 (54 down-regulated, 135 up-regulated) (Figure3A). Venn diagram analysis indicated that 45 the differential metabolites were common to all three comparison groups (Figure3B).

The difference fold change in metabolite quantitative information in each group was compared in combination with the grouping of specific samples. We display the most different metabolites in comparison groups (Figure3C, D and E). Results, Z6 VS HC4, indicated that Cyanidin O-malonyl-malonylhexoside, O-Feruloyl 2-hydroxylcoumarin, Cyanidin O-acetylhexoside, Eriodictiol C-hexosyl-O-hexoside, Cyanidin 3-O-glucoside (Kuromanin), Biochanin A, Myricetin, Quercetin 5-O-malonylhexosyl-hexoside, Cyanidin 3-O-glucosyl-malonylglucoside and Procyanidin A1 were Most expressed metabolite in, which belong Anthocyanins, Coumarins, Flavone C-glycosides, the class, Isoflavone, Proanthocyanidins. Tryptamine derivatives, Isoflavone, Anthocyanins, Amino acid derivatives, Flavone C-glycosides, Flavonol, Flavone, Coumarins, Benzoic acid derivatives, was most expressed metabolite in HC4. Corresponding results of other comparison groups was listed in Table1s, Table2s and Talbe3s of Supplementary material.

In order to explore the expression trends of Metabolites in different groups, we then performed a heat map analysis. Based on the relative differences, Metabolites resulted in three main clusters based on the relative in accumulation patterns in three groups (Figure 4A). Metabolites belonging to clusters 1Were higher quantity in HC4, while the Metabolites within cluster 3 and 4 were higher levels in z27 and z6 respectively. We then compared trend change of differential metabolite in any two groups. The differential metabolite obviously differentiated between between Z27 and HC4, 240 between Z6 and HC4, between Z6 and Z27 (Figure4B, Ffigure4C, Figure4D).

KEGG (Kyoto Encyclopedia of Genes and Genomesconduce) was conducted to integrate genes, expressions, and metabolites as a whole for research. The results of the annotation of the significant metabolite KEGG are classified according to the type of pathway in KEGG. The classification diagram is shown (Figure 5). We found that more Differential metabolite were in metabolic pathways and biosynthesis of secondary metabolites.

Discussion

Three different cultivars was planted in same time and place, however, many of agronomic trait, Seeding leaf color, Seed Color, of three sweet sorghum cultivars have a great change. Based on this, we use metabolomics to explore Mechanism of phenotypic change.

Plant metabolomics, as new direction in the post-genome era [19], reveal mechanism of change to metabolites in different tissues. Products of Metabolites was a response to genetic and environmental changes[20]. Therefore, metabolomic analysis is a very useful tool can to clarify the relationship between biological processes and phenotypes; furthermore, we can find some intuitive changes at the metabolic level[21]. Up until now, more and more reaches have reported to explore mechanism of the relationship between biological processes and phenotypes [5, 22]. In this study, we found 651 metabolite which was divided into 24 categories including Lipids_Fatty acids, Lipids_Glycerolipids, flavonoid, Benzoic acid derivatives, Anthocyanins, Nucleotide and its derivates, etc.(Table4s).

PCA and heat-map analyses of three groups showed a distinguished separation between the three cultivars. The results showed that there were large differences in the metabolites contained in the three groups. To further explore the mechanism of seed color differences between different cultivar, differentially expressed metabolites was analyzed between any two groups. There are 217 significantly different metabolites between Z27 and HC4, 240 between Z6 and HC4, 199 between Z6 and Z27. Although many substances, including Benzoic acid derivatives, Anthocyanins, Nucleotide and its derivates, have different variations between any two groups, the most different metabolites in comparison groups mainly are Anthocyanins and flavonoid. It is consistent with many reports [23, 24]. As we all known, anthocyanins are a kind of flavonoids, which are water-soluble plant pigments, which are the main causes of different colors such as white, red, black and blue in vegetables, seeds, fruits and flowers. Anthocyanins are very useful for plants, and they can prevent damage from harsh environments such as cold and drought, avoiding damage from fungi, bacteria and viruses, and beneficial to pollination and seed dispersal [25-27].

After get differentially expressed metabolites, the differential metabolites between three cultivars were mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database in order to obtain detailed pathway information. Results show that there are more differential metabolites were in metabolic pathways and biosynthesis of secondary metabolites. Secondary metabolites are mainly related to changes in the color of flowers and seeds, and also have a great effect on resistance to external aggression. The results also confirm this.

With the development of the technology of the sequencing, metabolomics method has been established and widely applied in much more research including Black Sesame and so on [28, 29]. Many new metabolites have been detected and characterized. In our research, 651 metabolites were divided into 24 categories, was found in Sweet Sorghum.

Sweet sorghum is cultivated throughout China, but it is mostly in the south of the Yellow River Basin. As the living standards of urban and rural people increase, it is no longer a staple food. Sweet sorghum is mainly used as feed in many countries such as China and Australia. Due to its high fiber density and homogenous flakes, it is well suited for use as a raw material for papermaking. It is also a new type of renewable and efficient energy crop [30, 31].

Conclusion

This study focused on the metabolic Diversity of three Sweet sorghum cultivars with white, red, And purple seeds to elucidate the factors responsible for the differences in Seed color. PCA and heat-map analyses of three groups showed a distinguished separation between the three cultivars. It shows that metabolites of three groups are quite different. Differentially expressed metabolites was Analyzed between any two groups and the most different metabolites in comparison groups mainly are anthocyanins and flavonoid. These findings have improved our understanding of the metabolic mechanisms accounting for differences in seeds color in different Sweet sorghum cultivars.

Abbreviations

PCA, principal component analysis; OPLS-DA, orthogonal signal correction and partial least squares-discriminant analysis; MRM, multiple-reaction monitoring; PLS-DA, partial least squares-discriminant analysis; SE, sensitivity; SP, specificity; KEGG, Kyoto Encyclopaedia of Genes and Genomes; LIT, Linear Ion Trap; QQQ, triple quadrupole; CAD, collision gas

Declarations

Ethics approval and consent to participate

This study did not involve any endangered or protected species and followed all relevant ethical guideline. The samples examined in this study were used as agricultural plant in China.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declare that they have no financial or other conflicts of interest in relation to this research and its publication.

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Authors' contributions

WZ as the corresponding author contributed to the conception of the study; YZ as the first author contributed significantly to analysis and manuscript preparation; GZ as the second author performed the data analyses and helped to write the manuscript; YL as the third author wrote the manuscript and helped to prepare the material; ZL and HL as the fourth and fifth authors helped perform the analysis with constructive discussions. All authors read and approved the final manuscript.

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Tables

Table 1. Sweet sorghum sorghum agronomic traits statistics table

name	Bud sheath color	Seeding leaf color	Tiller	Plant height (cm)	Stem dismeter (cm)	Main spike length (cm)	Main shank length (cm)
Z6	Green	Green	No	240	1.4	18	30
Z27	Purple	Purple	No	289	1.2	28	45
HC4	Green	Green	No	298	1.4	25	55

name	Spike out	Main pulse color	Spike shape	Shell color	Peridium rate	Seed Color	Seed shape
Z6	Short	white	Cattle heart	Black	1/2	white	Circle
Z27	Long	white	Clavate	Black	1/2	Red	oval
HC4	Medium	white	proculiform	Black	1/2	Black	oval

name	Grain weight (g)	Dry grain weight(g)	Cutin rate (%)	Shelling rate (%)	Grain uniformity	Stem and leaf senescence	sowing time
Z6	36	21	25	4%	tidiness	No	5.8
Z27	25	24	30	2%	tidiness	No	5.8
HC4	45	22	50	5%	tidiness	No	5.8

name	Sprout	Heading	Sprout-heading days	flowering	Sprout-flowering days	mature	Full growth period (days)
Z6	5.22	7.26	65	7.28	67	8.28	98
Z27	5.22	7.17	56	7.19	58	8.19	89
HC4	5.22	7.17	56	7.20	59	8.21	91

Figures

Figure 1

Seed color of the three sweet sorghum cultivars

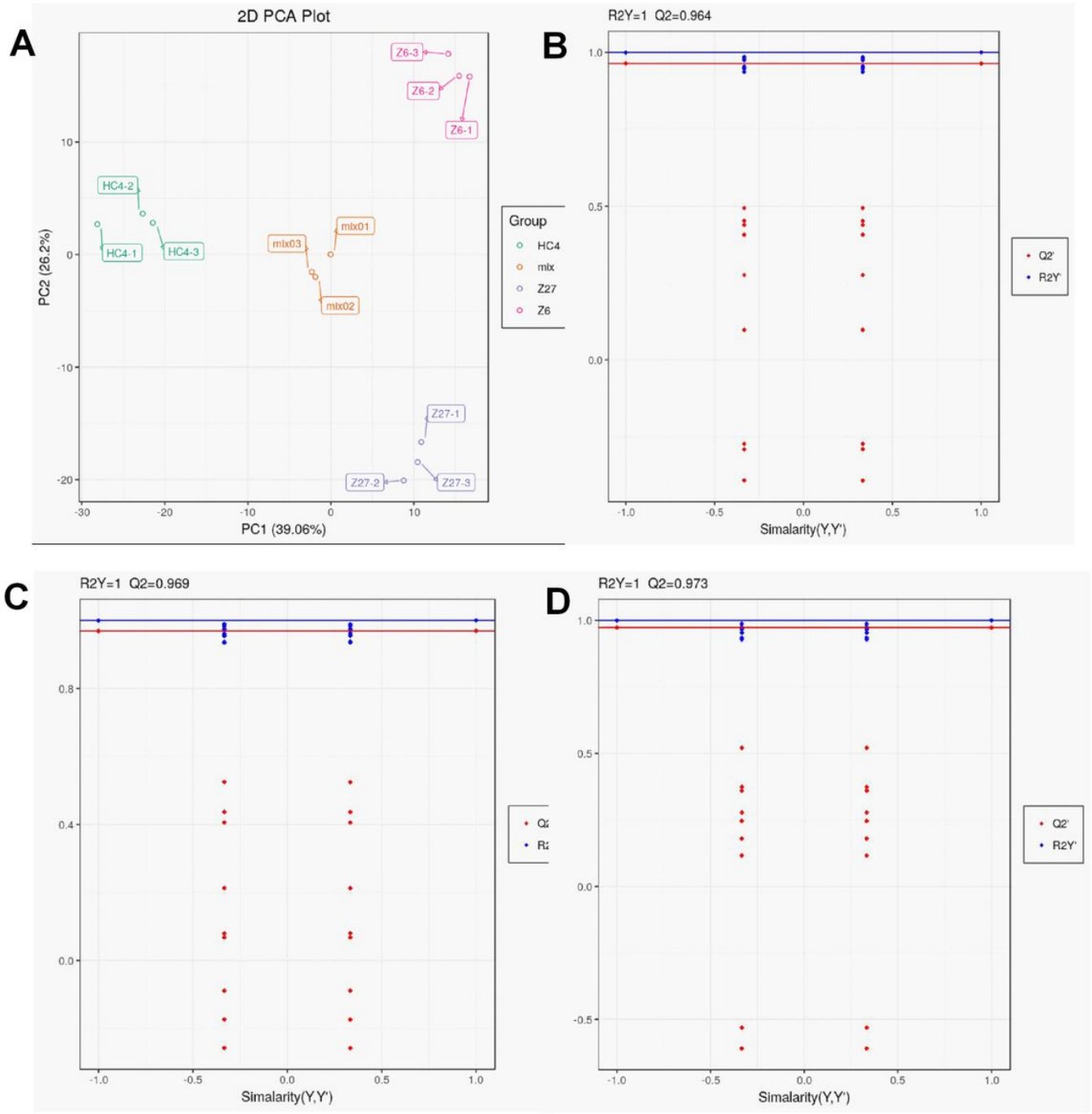

Figure 2

OPLS-DA and PCA. (A) PCA score plot. (B) OPLS-DA model plots for the comparison group between Z6 and Z27 (C) between Z6 and HC4 and (D) Z27 and HC4.

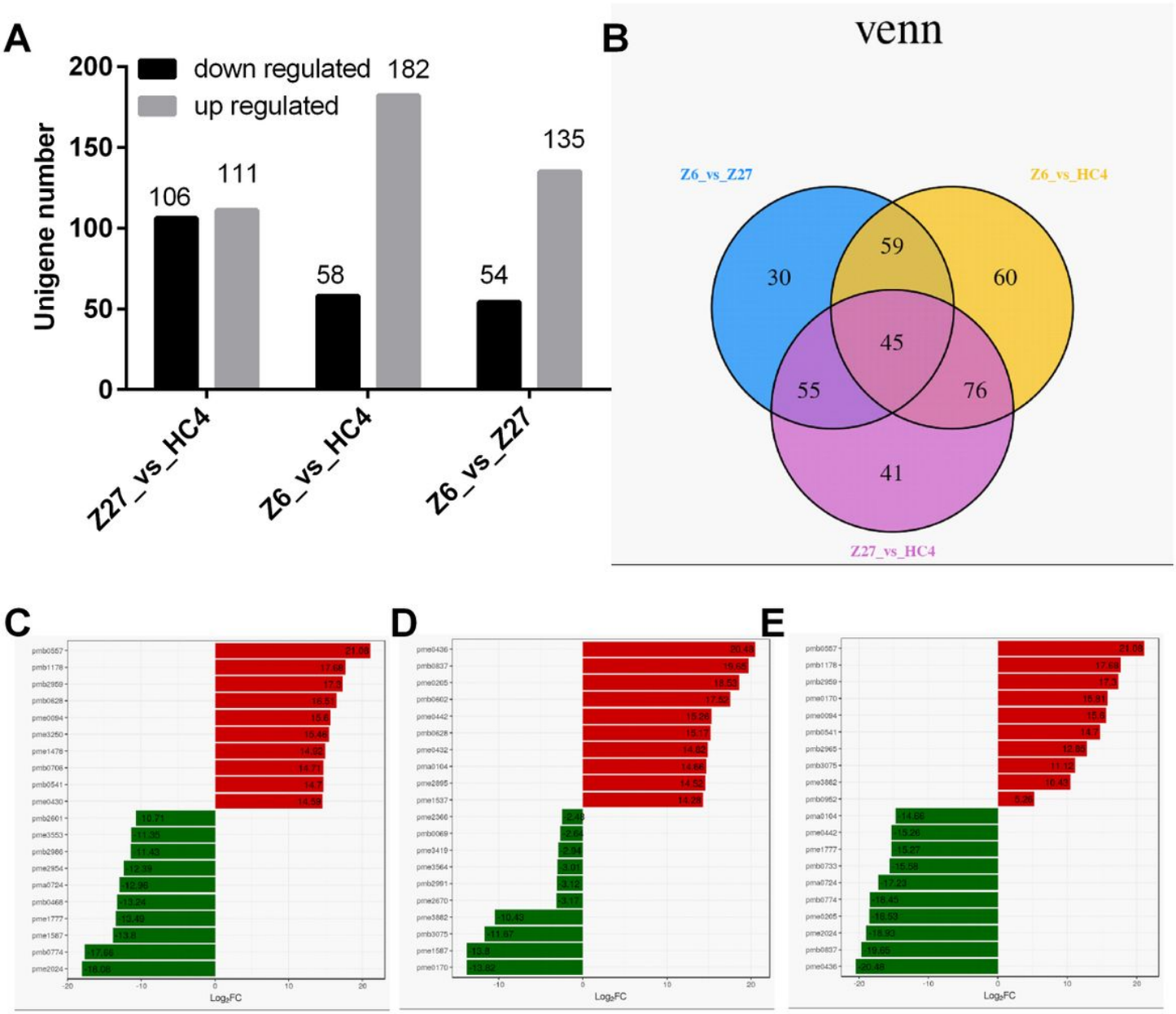

Figure 3

Numbers of significantly differentially accumulated metabolites,Venn diagram and 10 most different metabolites in comparison groups. (A) DAMs are shown in light color (upregulated) and black (downregulated) between Z27and HC4, between Z6 and HC4, between Z6 and Z27, respectively. (B) Venn Diagram result between three groups.(D-F) 10 most different metabolites in comparison groups between Z27 and HC4, between Z6 and HC4, between Z6 and Z27 in red color (upregulated) and blue (downregulated).

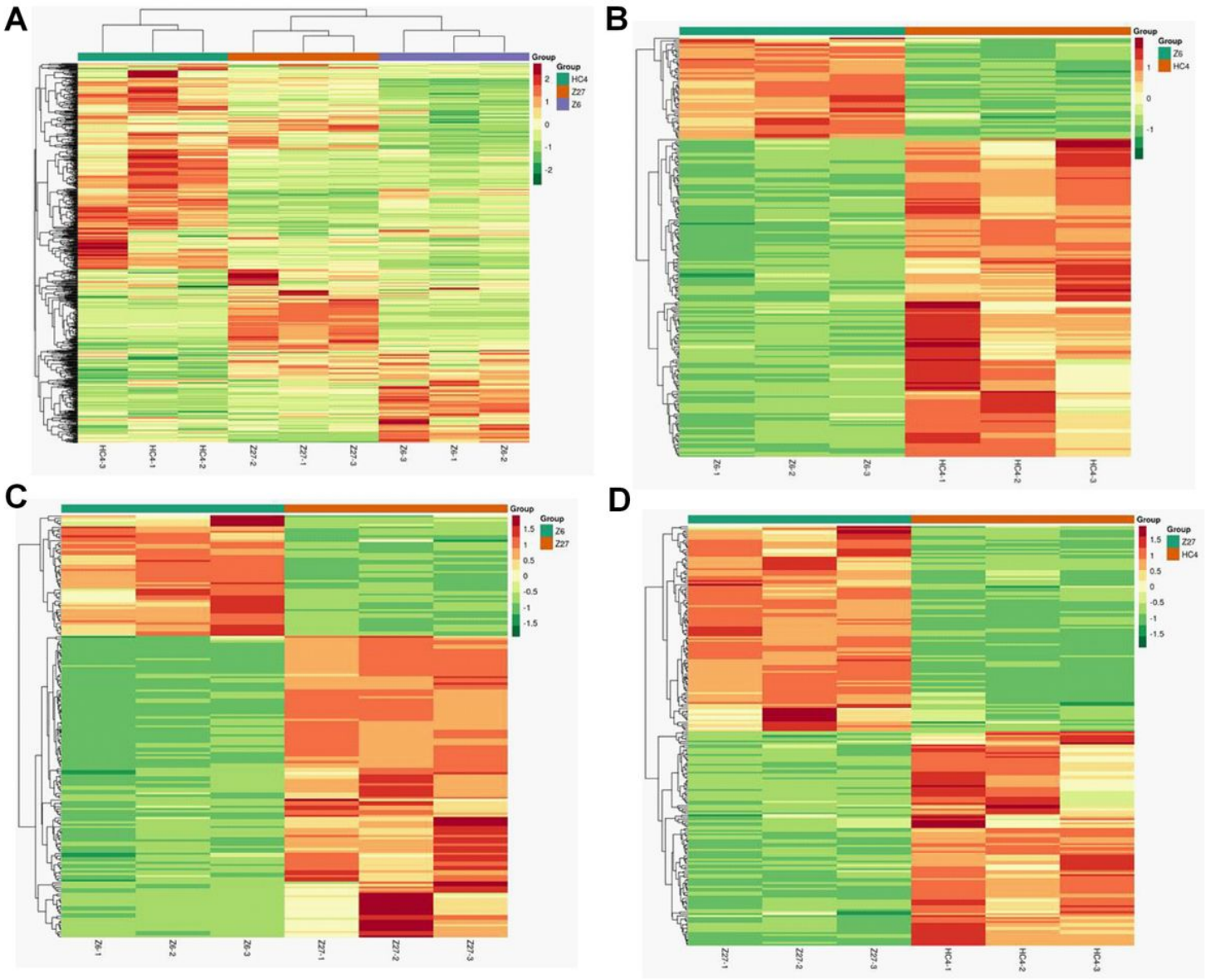

Figure 4

Clustering heat map of all metabolites and the differential metabolites heat map of the comparison group. (A) The heat map of all metabolites. (B) The differential metabolites heat map of the comparison group between Z6 and HC4, (C) between Z6 and Z27, and (D) between Z27and HC4.

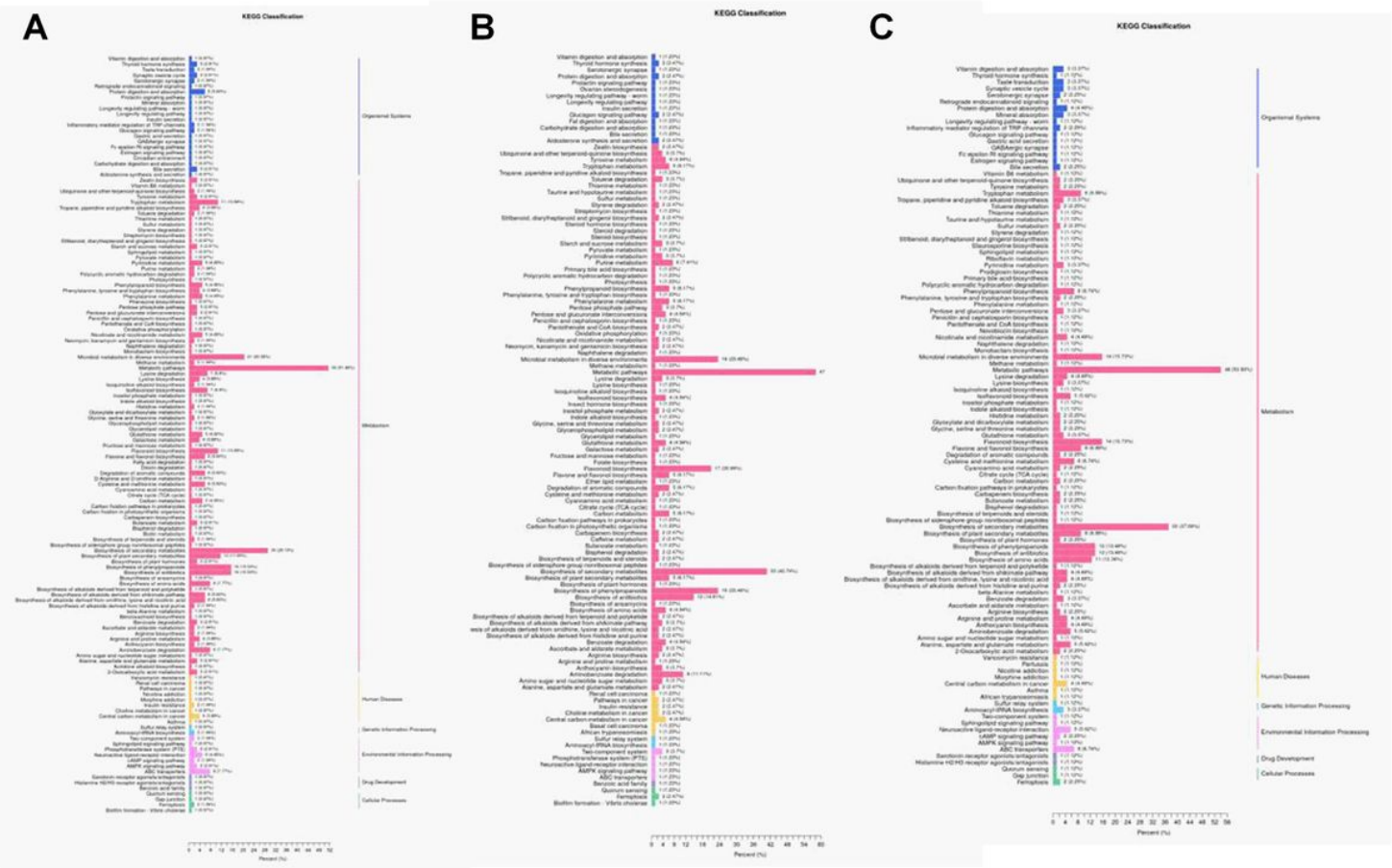

Figure 5

KEGG classification results. (A) The differential metabolites KEGG classification of the comparison group between Z27 and HC4, (B) between Z6 and HC4, and (C) between Z6 and Z27.

Supplementary Files

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