

Metabolomics reveals the defense mechanism of different rice under space flight stress

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Abstract

Background

Space flight is a special abiotic stress condition. Due to the development of space technology, its potential value has received widespread attention. Space mutation breeding is one of the important methods for human beings to solve food security. However, the molecular mechanism of space mutagenesis is still not very clear.

Result

In this study, two kinds of rice, Dongnong423 (DN3) and Dongnong416 (DN6), were carried on the SJ-10 retractable satellite for 12.5 days in orbit, returned to the ground and planted in the field until the three-leaf (TLP) and tillering stage (TS). The results of antioxidant enzyme activity, soluble sugar, and electron leakage rate revealed that the space environment caused the stress response of rice. The TLP and TS of DN3 identified 113 and 58 different metabolites, respectively. The TLP and TS of DN6 identified 107 and 77 different metabolites, respectively. These metabolites include amino acids, sugars, fatty acids, organic acids and secondary metabolites. We used qRT-PCR technology to explore the changes of enzyme genes in the TCA cycle and amino acid metabolism pathway. Combined with the results of metabolomics, we determined that during the TLP, the TCA cycle rate of DN3 was inhibited and amino acid metabolism was activated, while the TCA cycle rate of DN6 was activated and amino acid metabolism was inhibited. In TS, the TCA cycle rate of DN3 was inhibited, and amino acid metabolism was not significantly changed, while the TCA cycle rate of DN6 was activated and amino acid metabolism was inhibited. These results suggest that the space environment has different stress response mechanisms to the two rice varieties, and these differences may be reflected in energy consumption and compound biosynthesis.

Conclusions

This research uses metabolomics for the first time to explore the effects of space flight team rice. This research provides new insights for further exploring the effects of space biology and space mutation breeding.

1. Background

The development of space science and technology as well as the development and utilization of the space field have promoted the development of space life science. In the past few decades, rice, Arabidopsis, corn, fruit flies, and mice have become the main model organisms for studying space life science. The space environment mainly includes factors such as microgravity, radiation, circadian rhythm disturbance, weak magnetic field, and low temperature (Buchheim et al. 2019) . Therefore, the space environment is considered to be an extreme environmental condition. Previous studies have proved that plants can adapt to the space environment by reshaping their own metabolic network (Choi et al. 2019,

Barker et al. 2020), which provides the possibility for humans to use plants as a reliable food source in future long-term space missions.

In the process of space flight, plants will be subjected to extremely severe abiotic stress (Yurkevich et al. 2018), which will lead to reprogramming of the plant's genome, thereby affecting plant growth, development and yield (Musgrave and Kuang 2003, De Micco et al. 2014). Previous studies have indicated that the cell wall composition of *Arabidopsis thaliana* changes in the space environment (Hoson et al. 2014). At the same time, the results of transcriptomic revealed that the oxidative stress signal pathway of *Arabidopsis thaliana* was significantly changed in the space environment, and the oxidative stress signal was used to induce the expression of molecular chaperones to protect cells from oxidative damage (Choi et al. 2019). After the *Eruca sativa* seeds that stayed on the International Space Station for six months returned to the ground for planting, the emergence and growth of the seedlings were slightly delayed, and the senescence sensitivity was increased. Moreover, the transcript abundance of genes related to transcription and translation processes were reduced (Chandler et al. 2020). Other studies have proved that changes in barley leaf color and photosynthesis rate after space flight were related to chloroplast mutations (Jiayu et al. 2018). These results showed that the space environment can affect the growth and development of plants. But unfortunately, the orbital height and inclination of the aircraft were different during each space flight, which may cause certain differences in results. However, although the conditions and methods were different each time, it has been found that there were some overlapping biochemical pathways in spaceflight, which were regulated differently. These included pathways that involving heat shock, stress response, cell wall modification and cell defense (Johnson et al. 2017). In addition, the current research on how plants respond to the space environment was mostly based on transcriptomics and proteomics, while the results based on metabolomics have rarely been reported.

Rice (*Oryza sativa L.*) is one of the most necessary cereal crops all over the world, and nearly half of the world's population uses it as a staple food (Shen et al. 2018). It is also a model plant for molecular biology research (Chen et al. 2019). Many previous studies have reported how rice responds to the space environment, which has promoted our understanding of the effects of space biology and mutation breeding. In previous studies, changes in rice plant height, panicle length, tiller number and yield have all been found after space flight (Yu et al. 2007, Ma et al. 2007). In the STS-95 mission, it was found that under the stimulation of the space environment, the cellulose in the roots, matrix polysaccharides and root growth of rice changed (Hoson et al. 2003). Our reported that space flight led to changes in DNA methylation and gene expression, and some changes can be passed on to offspring, causing transgenic effects (Ou et al. 2009). Chen and Wang used the method of proteomics to explain the change mechanism of rice photosynthesis after space flight (Chen and Wang 2016). In our previous research, we found through proteomics that the two rice varieties (Dong nong 423 and Dong nong 416) had different responses to the space environment in different stages of rice growth (Zeng et al. 2020, Deyong et al. 2020). After space flight, the plant height of Dong nong 423 (DN3) of rice at the three-leaf stage was significantly higher than that of the control group, while Dong nong 416 (DN6) showed the opposite trend. However, the plant heights of these two types of rice at the tillering stage were not obviously different

from those of the control group. In addition, our research results also showed that space flight has caused differential expression of proteins involved in energy metabolism, signal transduction, gene transcription, protein synthesis, and protein modification in rice, and we believed that these changes may be induced by reactive oxygen species (ROS) (Zeng et al. 2020, Deyong et al. 2020). Our previous research also found changes in photosynthesis related processes (Cui et al. 2019). The current research on rice after space flight is based on genetic material, protein and phenotype. The complete study from the perspective of metabolomics has rarely been reported. As we all know that there are certain differences between the gene level and the protein level and the final metabolic level. Changes in the gene level and protein level cannot fully reflect the response mechanism of rice to the space environment. Therefore, the study of rice metabolomics in space environment is particularly important to reveal the mechanism of rice response space.

To clarify the complex process responsible for the defense mechanism of crops against the space environment at the metabolome level is extremely important for improving the revealing of the mechanism of space biological effects and the mechanism of space mutation breeding. In this study, we carried two different rice cultivars in space for 12.5 days through the practice of returning to the SJ-10 satellite. We used metabolomics methods to characterize the changes in the metabolites and basic regulatory pathways of two rice cultivars at different growth and development stages, and verified them with real-time fluorescent quantitative PCR. This work provides novel insights on how rice responds to the space environmental system at the metabolite level, further confirms that the space environment affects biomarkers of organisms, and contributes to the development of space mutation breeding.

2. Materials And Methods

2.1. Rice materials and spaceflight conditions

Seeds of two rice cultivars, DN423 (DN3) and DN416 (DN6) were provided by the Agricultural College of Northeast Agricultural University (Harbin, China). The spaceflight conditions described by Deyong et al (Deyong et al. 2020). Simply, two dry seeds of rice were carried on the SJ-10 retrievable satellite for 12.5 days for space flight treatment (SP-DN3, SP-DN6). The total radiation dose was 0.970 mGy (radiation equivalent = 160 μ Sv/d), and the gravity during flight was 10^{-4} g to 10^{-6} g. After returning to the ground, the seeds processed for space flight (SP-DN3, SP-DN6) and the control seeds (CK-DN3, CK-DN6) were planted in Wuchang City (Harbin, China).

Next, we randomly collected the leaves of the two rice cultivars at the TLP (TLP-CK3, TLP-SP3, TLP-CK6, TLP-SP6) and the TS (TS-CK3, TS-SP3, TS-CK6, TS-SP6), and stored them at -80°C for further analysis.

2.2 Plant weight measurement

At the TLP, the aboveground parts of 10 samples of two rice lines were taken to measure the plant weight and calculate the average plant weight. At the TS, the above-ground parts of three samples were taken

from each rice line to measure the plant weight and calculate the average plant weight. All operations were repeated three times.

2.3 Detection of electrolyte leakage rate

Use deionized water to wash the residues on the rice leaves, and then measure the electrolyte leakage rate (EL) according to the method described by Lutts et al. (1996). In short, put 0.5g of leaves into a 25ml test tube of deionized water, place it at 25°C for 3 hours and then measure the conductivity, which is recorded as M1. Next, boil the water bath for 10 minutes, cool to 25°C, measure the conductivity, and record it as M2. The EL was calculated according to the following formula:

$$EL = M1/M2 \times 100\%$$

2.4 Analysis of POD, CAT activity

Take 0.5 g of the sample into a glass homogenization tube, add 5 mL of phosphate buffer (0.1 mol / L, pH 7.4), and grind evenly in an ice bath. Centrifuge at 11000g for 15 minutes at 4°C. Then collect the supernatant and use it as crude enzyme extract for subsequent enzyme activity analysis. The activity of POD activity was analyzed according to the method of (Castillo et al. 1984). The activity of CAT activity was analyzed according to the method of (Aebi 1984).

2.5 Detection of soluble sugar content

The soluble sugar content was determined according to the anthrone colorimetry method. Take 0.1 g of the sample into a centrifuge tube, add 5 ml of 80% ethanol and extract in a water bath (80°C, 30 min), then centrifuge (6000 g, 5 min) and collect the supernatant. Repeat the extraction 3 times. Take the supernatant extract and add anthrone reagent, and measure the absorbance at 620 nm after 20 min of color development.

2.6 Extraction of metabolites and metabolomics analysis

The method of metabolomic analyses of the two rice cultivars was modified according to (De Vos et al. 2007). In brief, separately take 0.1g samples after grinding with liquid nitrogen and methanol: acetonitrile: water (1mL, 2:2:1, v/v/v) and mix them thoroughly, then perform low-temperature ultrasonic extraction (100W, 60min). After standing for 60 min (-20°C), centrifuge (4°C, 14000 g, 20 min), and take the supernatant and vacuum dry. Next, add 100 µL of acetonitrile: aqueous solution (1:1, v/v) to the dried sample for reconstitution, and centrifuge (4°C, 14000 g, 15min) to collect the supernatant. Immediately use high performance liquid chromatography (Agilent 1290, USA) tandem mass spectrometry (AB SCIEX 5500, USA) for analysis, and use positive ion and negative ion modes for detection. Partial least squares discriminant analysis (PLS-DA) and metabolic pathway analysis both use MetaboAnalyst 4.0 software (<http://www.metaboanalyst.ca/>). Principal component analysis (PCA) uses R software.

2.6 Total RNA Extraction and Real-Time PCR

Same as previous research (Deyong et al. 2020). Use TaKaRa kit (9767) to extract total RNA from rice leaves, and then use TaKaRa kit (RR073A) to transcribe RNA into cDNA. Follow the kit instructions. The gene expression level was analyzed according to the method described by (Cui et al. 2019). All primers are listed in Table S1.

3. Results

3.1 The change of morphological and physiological traits in two cultivars

In order to better understand the differences in the response of different rice strains to space flight at different growth and development stages, we analyzed the plant weight of rice and some physiological indicators of response to abiotic stress. Compared with the control group, the plant weight of the two rice cultivars changed during the TLP, while no difference at the TS (Fig.1A). Interestingly, the plant weight of the DN3 space flight group was significantly higher than that of the control group at the TLP, while the plant weight of DN6 was significantly lower than that of the control group at this stage (Fig.1A). We measured antioxidant enzyme activities, SSC and EI to evaluate the similarities and differences of the response of two different rice cultivars to the space environment (Fig.1B-E). In TLP and TS, the POD activity, CAT activity and EI in the two rice leaves treated by space flight were higher than the control (Fig.1B, C, E). In detail, in DN3, the activity of POD was significantly increased by 24.83% during TLP, while the activity of POD during TS increased, but there was no statistical significance ($P>0.05$). In DN6, the activity of POD increased by 23.67% and 18.08% in TLP and TS, respectively (Fig.1B). The activity of CAT increased by 22.3% and 32.2% in TLP and TS of DN3, respectively, and increased by 13.2% and 63.5% in TLP and TS of DN6, respectively (Fig.1C). In the two different growth stages of DN3 and DN6, EI increased by 51.9%, 35.5%, 37.4% and 28.2%, respectively (Fig.1C). We also characterized the changes of SSC after space flight. Space flight caused the content of SSC to decrease in DN3 TLP and increase in TS (Fig.1D). However, in DN6 we saw the opposite result with DN3 (Fig.1D). Based on the above results, it can be preliminarily determined that the space flight has caused changes in the physiological state of rice, and there were certain differences in the response of the two different rice lines to the space environment.

3.2 Space flight treatment globally reversed the metabolic profiles in rice

Metabolomics analysis was used to further reveal the impact of space flight on two different strains of rice. In the positive ion or negative ion mode, a clear separation between the CK group and the SP group was observed in the PCA mode of rice samples at different periods, which indicated that the space flight treatment can effectively change the metabolic characteristics of rice (Fig2 A-D).

In order to explore the main reasons for the differences in rice metabolites of different rice at different growth stages, we conducted partial least square discriminant analysis (PLS-DA). PLS-DA recognized 15 main components responsible for separation based on their VIP scores (Fig.3). These 15 metabolites revealed the main reasons for the separation of metabolites from each other in Fig.3. Obviously, 123-Benzenetriol, sucrose, tyramine, 2-oxoadipic, quinate, and 9(S)-HOTrE have the most significant changes

in the TLP stage of DN3. In the TS stage, nicotinamide, malvidin 3-o-glucoside cation, anthranilic acid, linolenic acid, and palmitic acid had the most significant changes (Fig.3.A D). The PLS-DA and VIP scores identified L-(-)-sorbitol, fructose, L-pyroglutamic, 9(S)-HOTrE, malvidin 3-o-glucoside cation, anthranilic acid, linolenic acid, and palmitic acid as dominant components for separation between the CK and SP6 (Fig.3.B E).

3.3. Space flight treatment altered the metabolite profiles in different biological samples of rice.

To understand the differences in the response of the two rice varieties to the space environment during different growth periods, we comprehensively considered the factor of change (FC), P value and VIP score. We defined metabolites with $FC \geq 1.2$ or $FC \leq 0.83$, $P \leq 0.05$ and VIP score ≥ 1 as differential metabolites (DEMs). The concentrations of most of the 113 (SP VS CK) metabolites in TLP-DN3 and 58 (SP VS CK) metabolites in TS-DN3 varied significantly after the space flight (Fig.4A). In TLP-DN6 and TS-DN6, there were 107 (SP VS CK) and 77 (SP VS CK) metabolites that changed significantly (Fig.4 A). In the DN3, 33 metabolites changed significantly in both TLP and TS stages (Fig.4B). However, only 10 metabolites among the 33 common metabolites maintained the same trend of changes in the two growth and development stages (Table.S2). 57 metabolites changed significantly in the TLP and TS stages of DN6 (Fig.4B). There were 19 metabolites that maintained the same trend of change in the two growth stages of DN6 (Table.S3). Moreover, the concentration of 16 metabolites changed significantly in the two growth and development stages of DN3 and DN6, which were listed in Fig.S1. In TLP-DN3, compared with the CK group, the concentration of L-Asparagine in the SP group increased by 38.93-fold, and the concentration of Nicotinamide decreased by 94% (Table.S2). The concentration of most metabolites in TS-DN3 increased significantly. What's more interesting was that most of the amino acids in TLP-DN3 have changed significantly but there was no change in TS-DN3 (Table.S2). The concentration of most metabolites in TLP-DN6 and TS-DN6 decreased, and the concentration of L-Asparagine and Maleamic acid decreased the most in TLP-DN6, reducing 98% and 98.3%, respectively (Table.S3). In addition, the number of differential metabolites at TS stage in DN3 and DN6 were less than that of TLP stage, which may imply that with the growth and development of rice, the influence of space environment on it was weakening.

3.3. Cluster analysis of space flight induced changes in metabolites

A correlation matrix was generated using Pearson correlation test to calculate the correlation coefficient between metabolites in order to study the interdependence of different metabolites. Correlation matrix revealed the relationship between different metabolites (Fig.5-8). Compared with TLP-CK3, TLP-SP3 except for aconitic acid and succinic acid, most of the organic acids (fumaric acid, citric acid, alpha-ketoglutarate) that involved in energy metabolism exhibited a negative correlation with amino acids (isoleucine, histidine, arginine, aspartate, glutamine, threonine, phenylalanine, tyrosine) (Fig. 5). It is worth mentioning that aconitic acid showed a positive correlation with arginine, aspartate, and tyrosine, but a negative correlation with histidine (Fig. 5). In addition, we noticed that succinic acid was positively correlated with histidine, and negatively correlated with phenylalanine and tyrosine (Fig. 5). The results in

Fig.5 also showed a positive correlation between changes in sugars (sucrose, mannose, raffinose) and most metabolites (Fig. 5). Compared with TS-CK3, pyruvic acid was negatively correlated with aconitic acid in TS-SP3, and it was positively correlated with fructose (Fig. 6). Acetyl-CoA, 1-palmitoylglycerol, sphinganine, l-kynurenine, biochanin A, 2-deoxyinosine, nicotinate ribonucleoside, deoxyguanosine, and vanillic acid showed a negative correlation. But it was positively correlated with rosmarinic acid, oxalosuccinic acid, n-Acetyl-l-leucine, s-(methyl) glutathione, N-acetylmethionine, L-pyroglutamic acid, and trans-3-Coumaric acid (Fig. 6). Glucose and tagatose were negatively correlated with enzyme N6-(lipoyl) lysine, glucose 1-phosphate, and fructose (Fig. 6). In addition, we haven't found any relevant changes in amino acid metabolism in Fig. 6. This showed that after space flight, DN3 had different metabolite profiles at different developmental stages. Similarly, we also analyzed the correlation between the metabolites in the two growth stages of DN6 (Fig. 7-8). Compared with TLP-CK6, pyruvic acid in TLP-SP3 was positively correlated with maleic acid, succinate, glutamine, valine, stearidonic acid, nicotinate, adenosine, dihydroxyacetone, and lactate (Fig. 7). Moreover, our results showed that most of the amino acids (aspartate, glutamine, proline, histidine, tryptophan, isoleucine, valine, leucine) and galactarate, limonene-1,2-epoxide, maleamic acid, kaempferol, ferulic acid, malic acid, glutamine and 2-hydroxyadenine showed a negative correlation (Fig. 7). Interestingly, the isoleucine in the space flight group and the control group showed a negative correlation (Fig. 7), which indicated that isoleucine in this period was seriously affected by space flight. In Fig. 8, 4-aminobutyric acid (GABA) is positively correlated with most compounds (argininosuccinate, mannose, sorbose, sucrose, 2'-deoxy-d-ribose, 2-hydroxyadenine, adenosine, glucose 1-phosphate, 2-deoxyribose 1-phosphate). Glutamine was positively related to a variety of amino acids (aspartate, histidine, arginine, lysine, homoglutamic acid) (Fig. 8). There was also a positive correlation between most amino acids and carbohydrates (Fig. 8). Our correlation analysis results revealed the metabolic relationship between organic acid metabolism, amino acid metabolism, and carbohydrates.

3.4 Analysis of metabolic pathways of two rice cultivars

MetaboAnalyst 4.0 software was used to analyze the metabolic pathways to better understand the impact of space flight on the biological processes of two rice cultivars, and the analysis results were displayed by bubble charts. The involved pathways were revealed in Fig. 9 and Fig. 10. Thirteen and eight metabolic pathways were significantly enriched in TLP-DN3 and TS-DN3, respectively (Fig. 9 A and B). These pathways mainly involved amino acid metabolism, TCA cycle, and pyruvate metabolism. There were 14 pathways that were significantly enriched in TLP-DN6 (Fig.10 A). They were: (1) TCA cycle; (2) Alanine, aspartate and glutamate metabolism; (3) Glyoxylate and dicarboxylate metabolism; (4) Aminoacyl-tRNA biosynthesis; (5) Galactose metabolism; (6) Valine, leucine and isoleucine biosynthesis; (7) Pyruvate metabolism; (8) Butanoate metabolism; (9) Glycolysis/Gluconeogenesis; (10) Linoleic acid metabolism; (11) Arginine biosynthesis; (12) Nicotinate and nicotinamide metabolism; (13) Phenylalanine, tyrosine and tryptophan biosynthesis; (14) C5-Branched dibasic acid metabolism (Fig.7 A). However, there were only 11 significant changes in TS-DN6 (Fig.6 B). They were (1) TCA cycle; (2) Alanine, aspartate and glutamate metabolism; (3) Aminoacyl-tRNA biosynthesis; (4) Arginine biosynthesis; (5) Galactose metabolism; (6) Glyoxylate and dicarboxylate metabolism; (7) Pyruvate

metabolism; (8) Glycolysis/Gluconeogenesis; (9) Phenylalanine, tyrosine biosynthesis, tyrosine and try metabolism; (11) Monobactam biosynthesis. Among them, amino acid metabolism, TCA cycle, glycolysis/gluconeogenesis, pyruvate metabolism, glyoxylate and dicarboxylate metabolism, galactose metabolism, aminoacyl-tRNA biosynthesis were all altered significantly for both growth stage (Fig.10). It is worth noting that pyruvate metabolism, TCA cycle, and amino acid metabolism were significantly enriched in the different growth and development stages of the two rice cultivars (Fig.9 and 10).

3.5 Expression of genes related to metabolic pathways

We noticed that two kinds of rice amino acid metabolism and TCA cycle were reshaped by the space environment. It is well known that the TCA cycle is significantly changed under stress to regulate the adaptability of plants to stress, and amino acids can be converted into intermediate products to compensate for the level of intermediate metabolites in TCA cycle. In addition, changes in the TCA cycle indicate that mitochondrial function is affected. Therefore, we explored the changes in genes related to the TCA cycle and amino acid metabolism (Fig.11, Table.S4). The expression of genes related to the TCA cycle showed different expression patterns at different growth stages of the two rice varieties. In DN3, the expression of most TCA cycle-related genes was down-regulated during TLP and up-regulated during TS (Fig.11A, Table.S4). DN6 showed the opposite trend with DN3. In DN6, the expression of TCA cycle-related genes was up-regulated during TLP, and down-regulated during TS (Fig.11A, Table.S4). The expression of genes related to amino acid metabolism also showed different expression patterns in different growth stages of the two rice varieties. In DN3, the expression of genes related to amino acid metabolism was up-regulated in TLP, and down-regulated in TS (Fig.11B, Table.S4). In DN6, the expression of genes related to amino acid metabolism during TLP was down-regulated, and the expression of TS was up-regulated (Fig.11B, Table.S4).

4. Discussion

Previous studies have reported that space flight affected the growth and development of different plants, such as *Arabidopsis thaliana* (Kranz 1986), rice (Yu et al. 2007), wheat (Mashinsky et al. 1994), *Tobacco* (Tepfer and Leach 2017), *Eruca sativa* (Chandler et al. 2020). In recent years, various omics methods have been used to study the effects of space flight on plants, but the application of metabolomics has not been reported. Our research reported the effects of space flight on the proteome of two different rice strains (DN6 and DN3) (Zeng et al. 2020, Deyong et al. 2020), and found that there were certain differences in their response to space flight. Here, we compared the changes in the metabolome profiles of two rice lines at different growth and development stages to reveal their metabolic responses to the spatial(Loreti et al. 2005) environment, and to study the chemical differences and conservation between species.

4.1 The effect of space flight on the overall morphology and physiology of two rice varieties

In this study, we observed that the plant weights of the two types of rice at the TLP showed different trends from that of the control group (Fig.1A), which was the same as the trend of rice plant height

changes we previously reported (Zeng et al. 2020, Deyong et al. 2020). As we all know, the changes in antioxidant enzyme activity reflected the biological redox state in plants (Mittler 2017). Our results showed that the activities of POD and CAT in rice were increased after space flight (Fig.1B, C), which indicated that the redox state in rice was disturbed at this time. Soluble sugar played an obvious central role in plant structure and metabolism (Couée et al. 2006). Moreover, the sugar signal produced by soluble sugar was connected in series with the ROS signal to control the redox state in the body (Contento et al. 2004, Loreti et al. 2005). The content of soluble sugar changed during the TLP and TS of two different rice varieties, and the changing trends were different (Fig.1D). This indicated that rice may adjust to sugar metabolism to cope with the effects of space flight, but there may be some differences in the mechanism. Electrolyte leakage rates is a sign of stress response in plant cells (Demidchik et al. 2014). Increases in electrolyte leakage rates have been reported in abiotic stresses such as heavy metals (Demidchik et al. 2003, Murphy and Taiz 1997), drought (Shcherbakova and Kacperska 1983), waterlogging (Shabala 2011), heat (Liu and Huang 2000), and oxidative stress (Demidchik et al. 2010). This study obtained the same changes as previously reported. The SP group of electrolyte leakage rates was higher than that of the CK group in the two types of rice (Fig.1E). Taken together, our results demonstrated that space flight has a stress response to two different strains of rice, and their response mechanisms to space stress may be different.

4.2 Space flight changes the process of TCA

The changes in plant mitochondrial function caused by space flight have been confirmed in previous studies (Sugimoto et al. 2014, Barker et al. 2020) . In this study, some intermediate products involved in the TCA have undergone significant changes (Table.S1). This indicated that space flight may have an impact on rice mitochondria, which further confirmed the conclusion of the changes in mitochondrial function shown in our proteomics results (Zeng et al. 2020, Deyong et al. 2020). In order to further confirm the influence of space flight on the TCA cycle of rice, we integrated the results of metabolomics and qRT-PCR to draw the TCA cycle metabolic pathway diagram (Fig.12).

The results of the transcriptome have confirmed that the space environment has induced changes in the Arabidopsis TAC cycle-related genes (Sugimoto et al. 2016), but the changes in the metabolic level of TCA were reported for the first time in this study. Under many abiotic stresses, TCA flux would change significantly (Chen et al. 2019). Therefore, TCA is generally considered to be an important physiological response that regulates the adaptability of plants to abiotic stress. In addition, the intermediate metabolites of the TCA cycle were precursors for the synthesis of amino acids, fatty acids and secondary metabolites (Chen et al. 2019, Kumari and Parida 2018). In our research, the content of aconitic acid, alpha-ketoglutarate, maleic acid, succinate, fumaric acid, pyruvic acid, citric acid, isocitric acid and other organic acids have changed significantly (Fig. 12).

In TLP-DN3 and TS-DN6, the content of most intermediate products in the TCA cycle was reduced, and the expression of enzyme genes encoding the enzymatic reaction in this pathway was down-regulated, which indicates that the TCA cycle rate in TLP-DN3 and TS-DN6 was reduced. However, in TLP-DN6 and

TS-DN3, the content of intermediate metabolites increased, and the expression of related genes related to enzymatic reactions was up-regulated, indicating that the TCA rate in TLP-DN6 and TS-DN3 increased. Therefore, the metabolic fluxes of the TCA cycle were different in the two different growth and development stages of the same rice strain (Fig. 12). What is more interesting is that the TCA cycle fluxes of the two types of rice at the same growth and development period were different, and the two types of rice show opposite trends (Fig. 12). Under salt stress, the content of pyruvate, oxalic acid, malic acid, citric acid and succinic acid in cosmos decreased (Kumari and Parida 2018). This decrease may be the decrease of TCA cycle activity and the increase of carbon structure, which was used to synthesize the compounds needed for stress response (Kiani-Pouya et al. 2017). In addition, under the stress of 2,2',4,4'-Tetrabromodiphenyl ether, the TCA cycle rate of rice increased, for the reason that 2,2',4,4'-Tetrabromodiphenyl ether will activate a series of reactions to provide energy for the normal life of rice (Chen et al. 2019). Therefore, the effects of stress conditions on the TCA cycle of plants may be different, which showed again in our research that there were differences in the mechanisms of the two types of rice in response to the spatial environment at different growth stages.

Citric acid is an important organic acid that participates in the absorption of iron ions by plants and numerous physiological and biochemical reactions (Hell and Stephan 2003). It is reported that citric acid involved in aluminum poisoning (Ma and Furukawa 2003), iron stress (Shlizerman et al. 2007), heavy metal stress tolerance (Gao et al. 2010), salt stress (Sun and Hong 2011). Therefore, the change in citric acid content in this study reflected the differences in the resistance of the two rice varieties to the space environment at different growth stages.

In our results, we also observed a significant increase in alpha-ketoglutarate in TLP-DN3, TLP-DN6 and TS-DN3. Isocitrate dehydrogenase can convert isocitrate into α -ketoglutarate through oxidative decarboxylation, so that plants can produce ATP. The content of isocitrate in TS-DN3 was consistent with the content of alpha-ketoglutarate, which indicated that the increase of alpha-ketoglutarate in TLP-DN3 may be caused by the change of isocitrate. It is worth noting that only one of the three genes encoding isocitrate dehydrogenase was up-regulated, and our proteomic results did not show increased abundance of isocitrate dehydrogenase (Deyong et al. 2020). This indicates that the activity of isocitrate dehydrogenase may be regulated. In addition, we also noticed that fumaric acid was increased in TLP-DN3, TLP-DN6, TS-DN3 and TS-DN6. The increase of fumaric acid helped to improve the tolerance of plants to stress conditions (Song et al. 2012).

Our results revealed that the TCA rates of the two rice varieties at different growth and development stages showed different patterns after space flight. We surmised that it was caused by the different response mechanisms of the two types of rice to the space environment.

4.3 Space flight changes the process of amino acid metabolism

Amino acids play a vital role in plants. They are the basic building blocks of proteins and provide necessary intermediate metabolites for many metabolic reactions. It is an important molecular form of plant organic nitrogen. Therefore, strict control of the biosynthesis, degradation and transportation of

amino acids can meet the needs of plants for nitrogen and carbon utilization (Pratelli and Pilot 2014). Amino acids can also act as antioxidants to respond to various abiotic stresses (Caldana et al. 2011). Here, we combined the results of metabonomic and qRT-PCR to map the complex network of amino acid transformation in rice at different growth and development stages after space flight to reveal the changes of amino acid metabolism in rice (Fig.13).

In TLP-DN3, there were 15 kinds of amino acids that showed a complex network relationship. Among these 15 kinds of amino acids, except for the decrease in glutamine content, the others all showed an increasing trend (Fig. 13 A). The expression of genes encoding these amino acid interconversion enzymes also showed up-regulation (Fig. 13 A), so we confirmed that amino acid metabolism was activated when DN3 developed into TLP after space flight. Interestingly, we did not find significant changes in these amino acids in TS-DN3 (Table S1), but qRT-PCR results showed a significant down regulation of the genes encoding the enzyme (Fig. 11B). This may also be one of the reasons why the amino acids of TS-DN3 have not changed significantly. Moreover, it also suggested that TS-DN3 may adopt a simpler biosynthesis pathway to cope with the impact of space environment. For DN6, the amino acid content was reduced during TLP (Fig. 13 B), and the expression of enzyme genes was mostly down-regulated (Fig. 13 B), while the content of amino acids increased in TS (Fig.13C), and the expression of enzyme gene was up-regulated (Fig.13C). Therefore, after space flight, the amino acid metabolism in TLP-DN6 was inhibited while the amino acid metabolism in TS-DN6 was activated. Like the TCA cycle, amino acid metabolism shows different changes in different growth and development stages of the same variety, and different changes in different varieties in the same growth and development stage. It is worth noting that amino acid metabolism and TCA cycle showed opposite activation or inhibition patterns in different rice at the same period, which indicates that there may be a certain balance between amino acid metabolism and TCA cycle. The intermediate metabolites in TCA cycle can be converted to some amino acids, resulting in the decrease of intermediate metabolites in TCA cycle. When the intermediate metabolites in TCA cycle decrease, amino acids can be converted into key products of TCA cycle under the catalysis of enzymes to ensure the rate of TCA cycle. This reasonably explains the main reason for the opposite change between amino acid metabolism and TCA cycle in this study.

Glutamic acid, proline and arginine can be converted into alpha-ketoglutarate (Fig.13), which affects the reaction activity of TCA. It also explains that the content of alpha-ketoglutarate increased in TLP-DN3 when the intermediate metabolites of TCA cycle were reduced (Fig.12 A). Proline has been proved to respond to abiotic stresses. The catabolism of proline in mitochondria can transfer electrons to respiratory chain to promote the production of ROS and ATP, and this process was intensified under drought stress (Liang et al. 2013, Schertl and Braun 2014). But when plants grow in adversity for a long time, they can quickly remove excess proline by increasing the amount of catabolic enzymes, and prevent invalid circulation by post translational regulation (Batista-Silva et al. 2019). This explains the difference of proline content between the two rice varieties in different growth and development stages, as well as the difference of ROS in previous work. On the other hand, it also shows that there are differences in the mechanism of response to spatial environment between the two rice varieties.

Glutamate decarboxylase catalyzes the decarboxylation of glutamate to produce GABA. GABA in plants can respond to biotic and abiotic stresses such as temperature, dehydration, salinity, oxygen stress, mechanical damage, acidosis, and virus infection (Bouché and Fromm 2004). GABA is also a signaling molecule. It can participate in the control of C: N balance in plants through direct interaction with glutamate receptors (signaling) or by controlling the utilization of glutamate (metabolic) (Kang and Turano 2003). Recent studies have shown that GABA can affect the development of plant roots and can negatively regulate the content of malic acid in plants (Ramesh et al. 2015). In our study, GABA content changed significantly in TLP-DN6 and TS-DN6. Therefore, this may also be one of the reasons for the changes in their malic acid content, which also showed that GABA can affect the activity of TCA by adjusting the malic acid content.

4.4 The effect of space flight on other metabolic reactions of two kinds of rice

Soluble sugars especially sucrose, glucose and fructose play a central role in maintaining plant cell structure and metabolism. They are involved in the response to various abiotic stresses, acting as signal molecules for nutrients and metabolites and activating or interacting with specific plant hormone transmission pathways, resulting in changes in gene expression and protein abundance (Couée et al. 2006). The changes of these sugars were also found in our metabolome results (Table.S1 and Table.S2). Compared with the TS-CK3, fructose and glucose increased by 2.4 times and 1.9 times respectively in the TS-SP3 while the sucrose content did not change, but the sucrose content of these three sugars have only reduced by 83% in TLP-DN3 (Table.S1). The content of these three sugars in the TS-SP6 was significantly lower than that of the TS-CK6, while only the sucrose content in TLP-SP6 increased by 2.4 times, and the concentration of the other two sugars decreased (Table.S2). Previous studies have found that changes in sugar concentration showed unique expression patterns under different abiotic stresses and in different species. For example, drought stress, salt stress, and low temperature stress will increase sugar concentration, while heavy metals, malnutrition, and ozone will reduce sugar concentration (Rosa et al. 2004, Gill et al. 2003, Prado et al. 2000, Rosa et al. 2009). These results proved that the space flight caused the response of rice to stress, and the response pattern of the two types of rice was different. Other studies have shown that the sugar accumulation caused by the sugar disturbance caused by the stress response will also limit the growth of rice seedlings (Mishra and Dubey 2013). This may also be the reason for the inconsistent plant weights of the two rice plants in our study. Our research also found some other carbohydrate changes (Table.S1 and Table.S2). Mannose and raffinose can protect plant cells from oxidative damage caused by various stress conditions, which has been determined that they have the ability to eliminate ROS (Nishizawa et al. 2008). After space flight, mannose in DN3 and DN6 was accumulated in TLP and TS, while the concentration of raffinose decreased (Table.S1 and Table.S2). It is reported that drought stress (Shahbazy et al. 2020) and salt stress (Kumari and Parida 2018) have disturbed the changes of D-tagatose. Our results showed that D-tagatose in DN3 decreased in the TLP and TS stages after space flight, and was accumulated in the TS stage of DN6. Moreover the concentrations of glucose, sucrose, maltotriose, rhamnose, and raffinose had different trends in the two rice varieties after space flight. These evidences suggest that there is a differential metabolic rearrangement of carbohydrate metabolites in different varieties and different growth stages. In addition,

our research also provided the change pattern of carbohydrate metabolites in rice after space flight for the first time.

Many studies have shown that vitamins can reduce the damage caused by abiotic stress to plants (Taffouo et al. 2009, Asensi-Fabado and Munné-Bosch 2010). Abiotic stress leads to a lack of vitamins and cofactors, which will reduce the growth performance of plants, and supplementing plants with the lack of vitamins under stress conditions will improve their growth performance (Hanson et al. 2016). Niacinamide is the common form of vitamin B3 (niacin), which is the active part of the coenzymes NAD(P) and NAD(P)H. It participates in the redox reaction of various enzymes and plays an important role in the energy metabolism of the organism (Hunt et al. 2004). Studies have proved that nicotinamide can respond to the oxidative stress response of UV-B to *Pisum sativum* (Berglund et al. 1996), and metabolize nicotinamide into hydrochloric acid when in plants. Furthermore, Niacinamide and hydrochloric acid can prevent DNA breakage and cell electrolyte leakage caused by oxidative stress, and induce the accumulation of glutathione, aconitase and fume at the same time (Berglund et al. 2017). Compared with the control group, nicotinamide decreased by 54% and 67% in TLP-DN3 and TS-DN3, respectively, but decreased by 94% in TLP-DN6, and increased by 1.57 times in TS-DN6 after space flight (Table.S1 and Table.S2). In addition, it was found that niacin had different trends in the TLP of DN3 and DN6 (Table.S1 and Table.S2), which may be caused by the different activities of nicotinamides in the two kinds of rice. This may also explain the changes in aconitase and fume content in our research. Pyridoxal and pyridoxine are two forms of VB6 (Czégény et al. 2019). Pyridoxine can regulate the tolerance of plants to abiotic stress and affect root development (Chen and Xiong 2005). Our study showed that pyridoxine reduced 37% in TLP-SP3, and accumulated 3.95 times and 3.08 times in TS-SP3 and TLP-SP6, respectively. Moreover, we also found that pyridoxal accumulated 2.53 times and 2.90 times in TLP-SP3 and TLP-SP6, respectively. Our results showed that the content of Pyridoxine increased when DN3 rice developed from TLP to TS after space flight, but it showed the opposite trend in DN6. These kinds of vitamins showed different trends in the two kinds of rice, which indicated that the vitamin metabolism of the two kinds of rice showed different expression patterns to the spatial environment. What's more exciting is that these vitamins can respond to the oxidative stress effect of abiotic stress. Therefore, our results implied that the space flight had an oxidative stress effect on the two kinds of rice, and this effect continued until the TS period of rice.

5. Conclusion

This study investigated the metabolic disturbances of different rice strains at different growth and development stages caused by space flight, and compared the molecular differences in the response of the two rice strains to the space environment. Our research showed that space flight caused changes in the metabolic profiles of two rice lines and caused rice stress responses. DN3 and DN6 adapted to changes in the space environment by remodeling metabolic pathways, but their mechanisms had certain differences. We observed that space flight changed the intermediate metabolites of TCA cycle and amino acid metabolism. We further confirmed the effect of space environment on TCA cycle and amino acid metabolism by using qRT-PCR and metabolomics results. Space flight resulted in the decrease of TCA

rate and increase of amino acid metabolism rate of DN3 at TLP. The TCA cycle rate of DN3 increased in TS while the amino acid metabolism rate did not change. This indicates that TLP took more complex metabolic regulation than TS in DN3 to adapt to the impact of space flight. In DN6, we observed the increase of TCA rate and the decrease of amino acid metabolism rate in TLP. However, the TCA was inhibited and the amino acid metabolism rate was increased when dn6 was treated with TS. These results provided useful insights into the molecular mechanism of different biological effects of space environment on rice, and new reference for space biological effect mechanism and space mutation breeding. Our research focuses on the impact of space environment on rice leaves, and the impact of space flight on rice grain quality and yield need further research.

Declarations

Ethics Approval and Consent to Participate

Not applicable

Availability of Data and Materials

Dataset and figures supporting the results are included as additional files

Authors' Contributions

Professor Weihong Lu and Professor Jie Cui conceived and designed the study. Deyong Zeng performed the experiments and wrote this manuscript. Professor Yeqin Sun and Shuanghong Guan provided the materials, Professor Dayou Cheng provided the experimental site. Yishu Yin, Yi Xiong and Mengyao Liu made structural and linguistic changes to the manuscript respectively. All authors read and approved the final manuscript.

Conflict of interest

The authors have no conflicts of interest to declare.

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Consent for Publication

All authors have provided consent for publication

Competing Interests

The authors declare that they have no competing interests.

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Figures

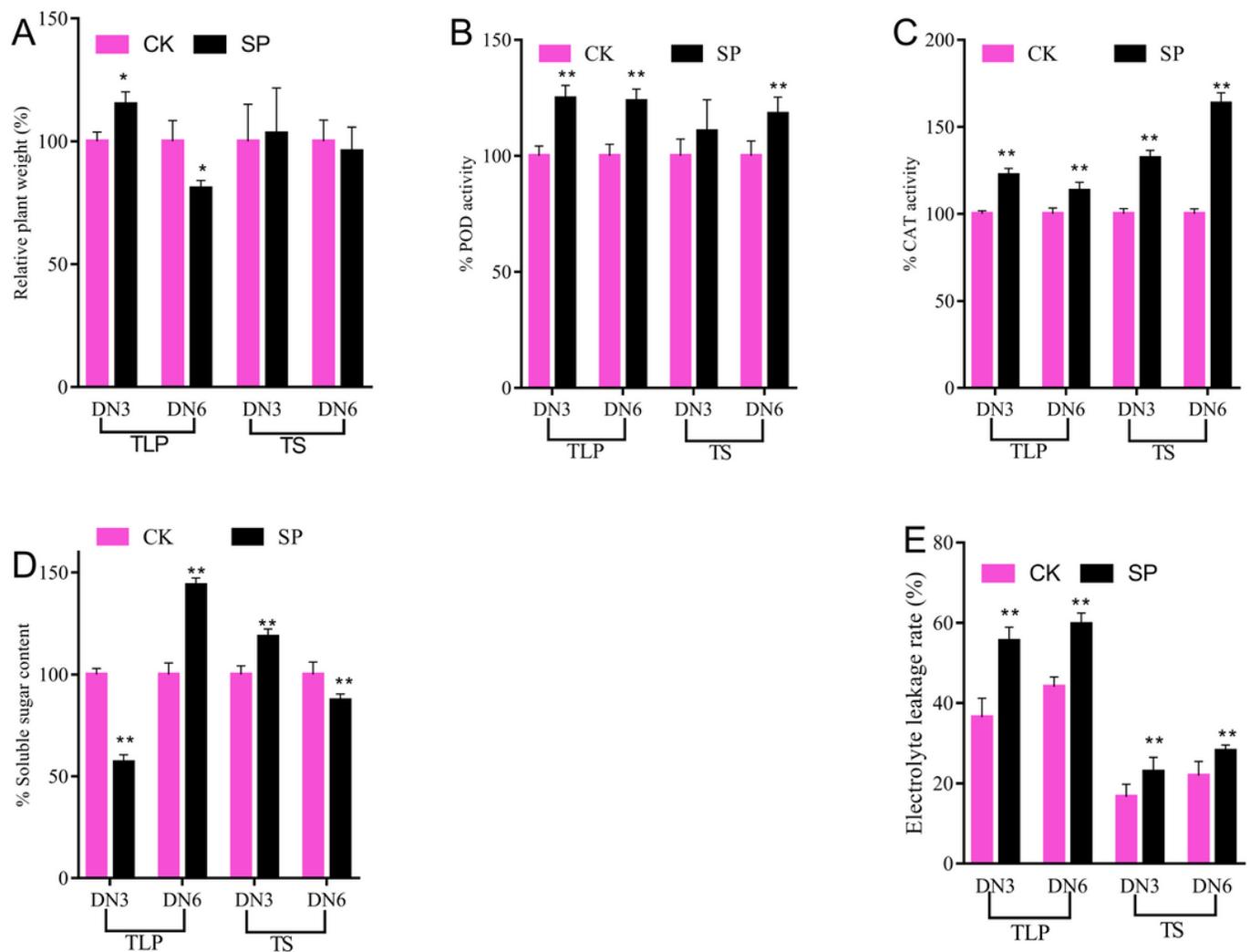


Figure 1

The morphological and physiological changes of rice induced by space flight. (A) Percent values of plant weight. (B) Percent values of POD activity. (C) Percent values of CAT activity. (D) Percent values of soluble sugar content. (E) the electrolyte leakage rate change. TLP, three-leaf stage; TS, tillering stage; red column represents the control group; black column represents space flight group. Data are expressed as mean \pm SD, n = 9, ** indicate significant difference at $p < 0.01$ by Student's t-test (GraphPad Prism 7), respectively.

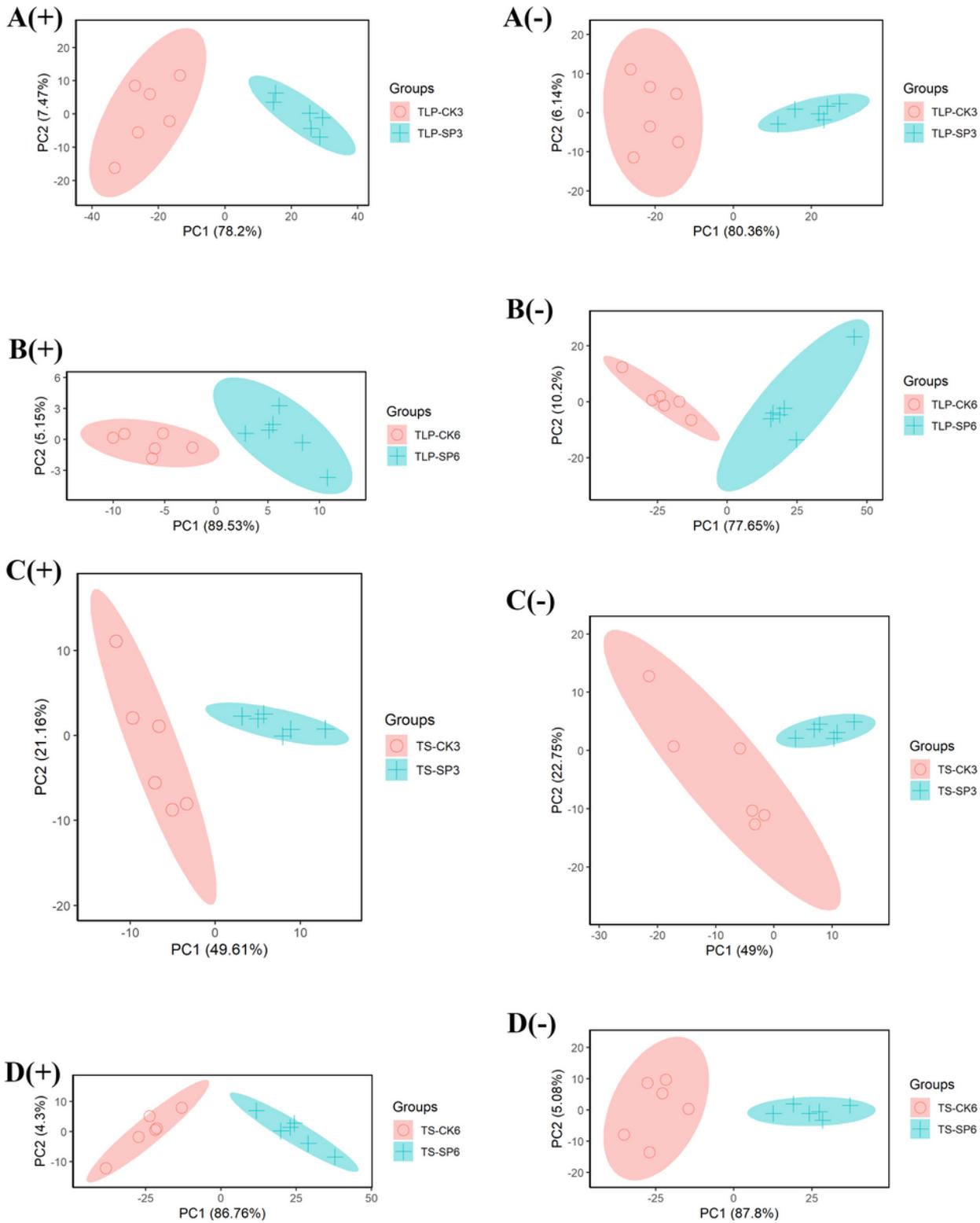


Figure 2

PCA score map of different rice sample groups based on UHPLC-QTOF/MS data. (A) Rice samples of DN3 at the TLP, (B) Rice samples of DN6 at the TLP, (C) Rice samples of DN3 at the TS, (D) Rice samples of DN6 at the TS; (+) and (-) represent positive and negative ion; TLP and TS represent three leaf period and tillering stage.

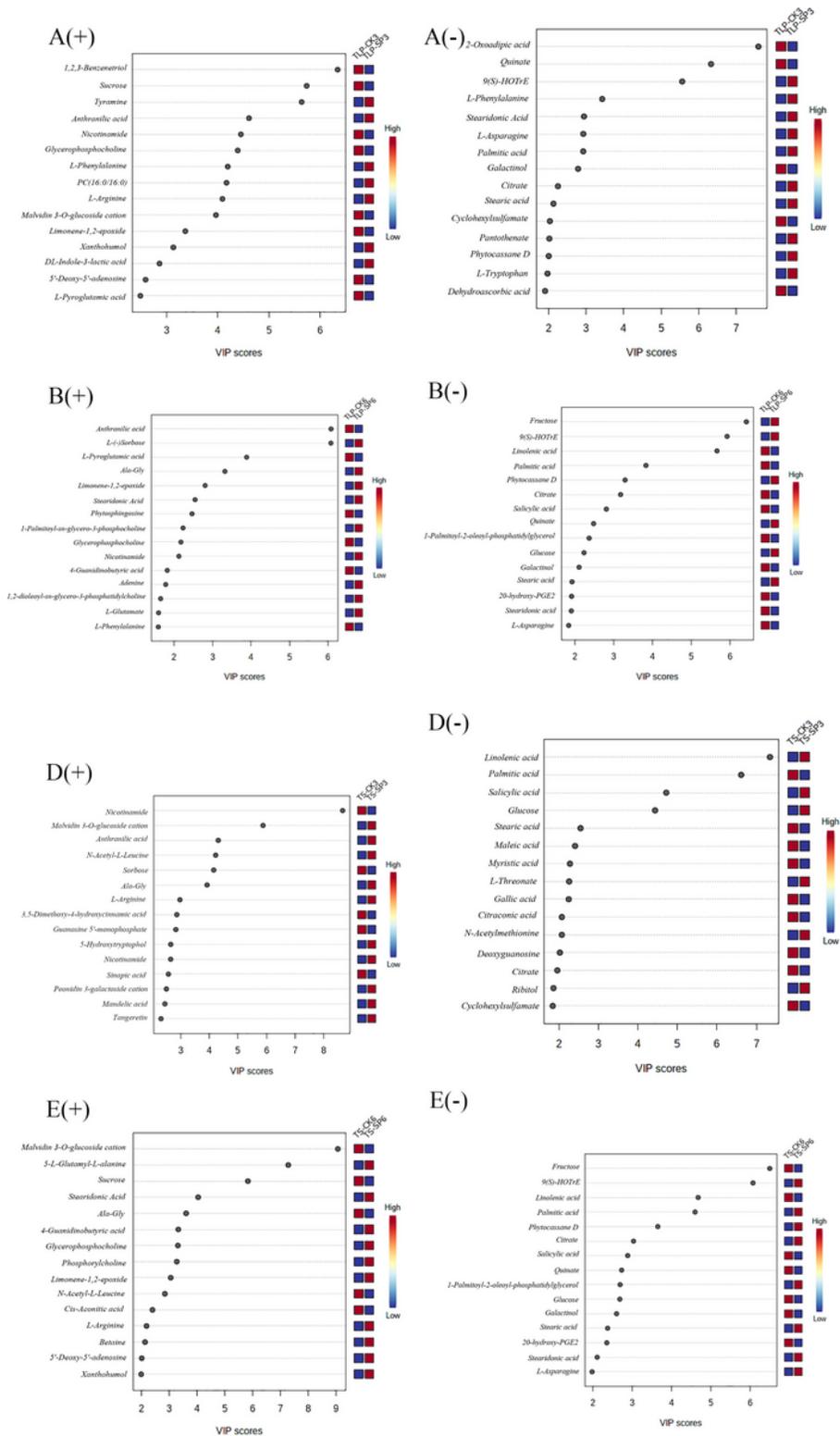


Figure 3

Variable importance in projection (VIP) scores of the top 15 metabolites in grains of the two rice cultivars in different growth stage. Results were from the partial least squares-discriminant analysis (PLS-DA). (A) Rice samples of DN3 at the TLP, (B) Rice samples of DN6 at the TLP, (C) Rice samples of DN3 at the TS, (D) Rice samples of DN6 at the TS; (+) and (-) represent positive and negative ion; TLP and TS represent three leaf period and tillering stage. N3 and N6 represent DN423 and DN416. The VIP scores

demonstrated the relative contribution of these metabolites to the separation among four concentrations. The higher the VIP scores, the larger the contributions. The relative contents of metabolites were displayed by the color boxes, with red indicating high and green indicating low contents.

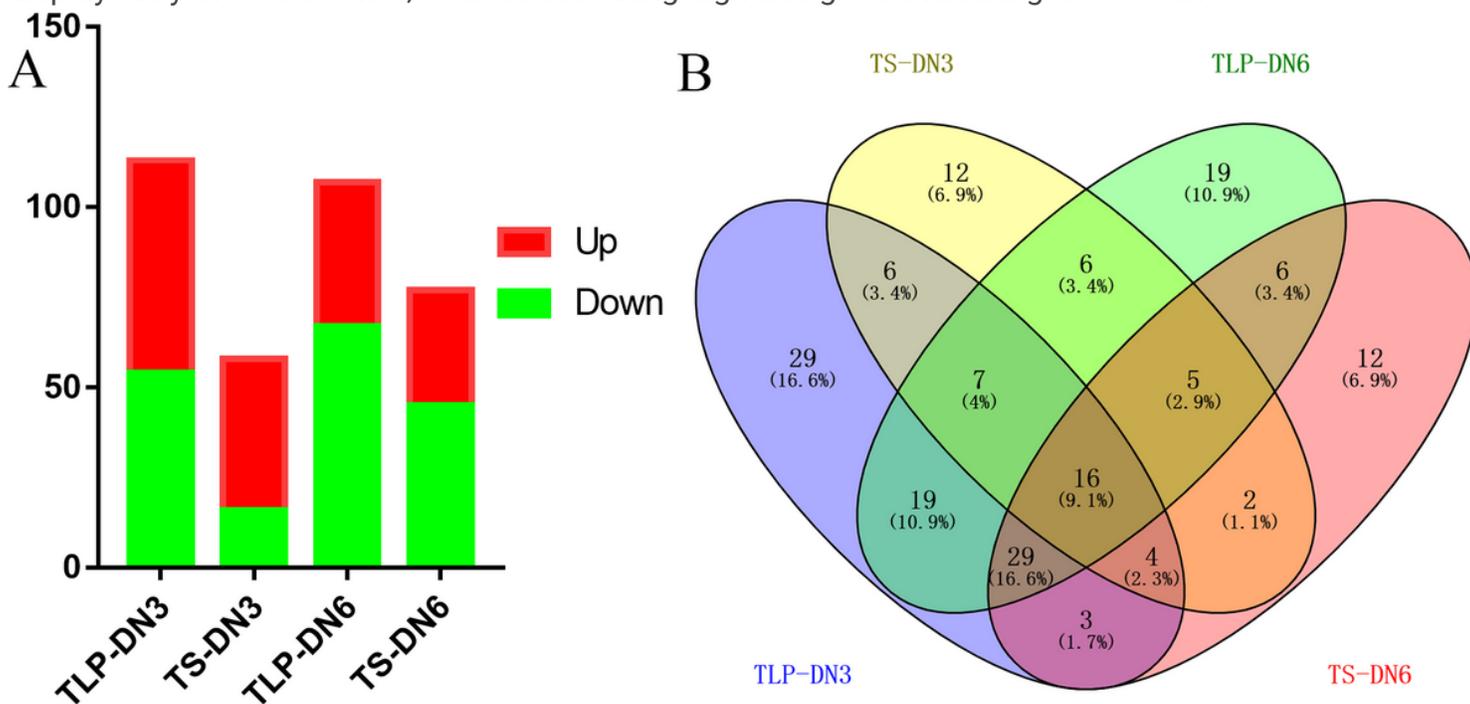


Figure 4

The number of up-regulated and down-regulated DEMs in two different growth stages (A). Venn diagram showed the overlaps of the DEMs in different rice (B).

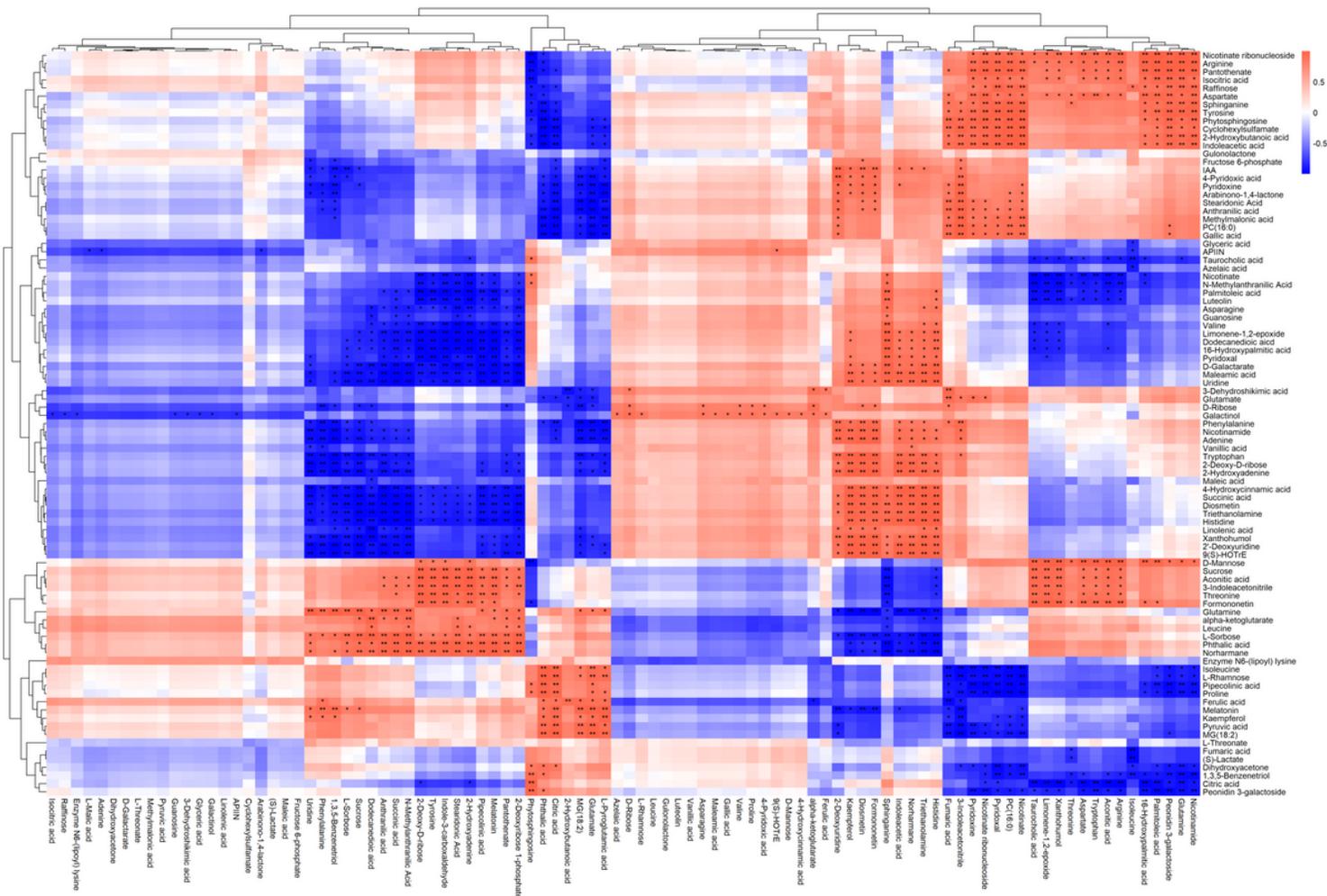


Figure 5

Correlation analysis of TLP-DN3 leaf metabolites subjected to space flight treatment. A correlation matrix was generated using Pearson's correlation on six biological replicates of CK and SP-treated plants. Red colour shows positive correlation whereas blue colour represents negative correlation among the metabolites under space flight treatment. ** and * indicate significant difference at $p < 0.01$ and $p < 0.05$ by Pearson's correlation test, respectively.

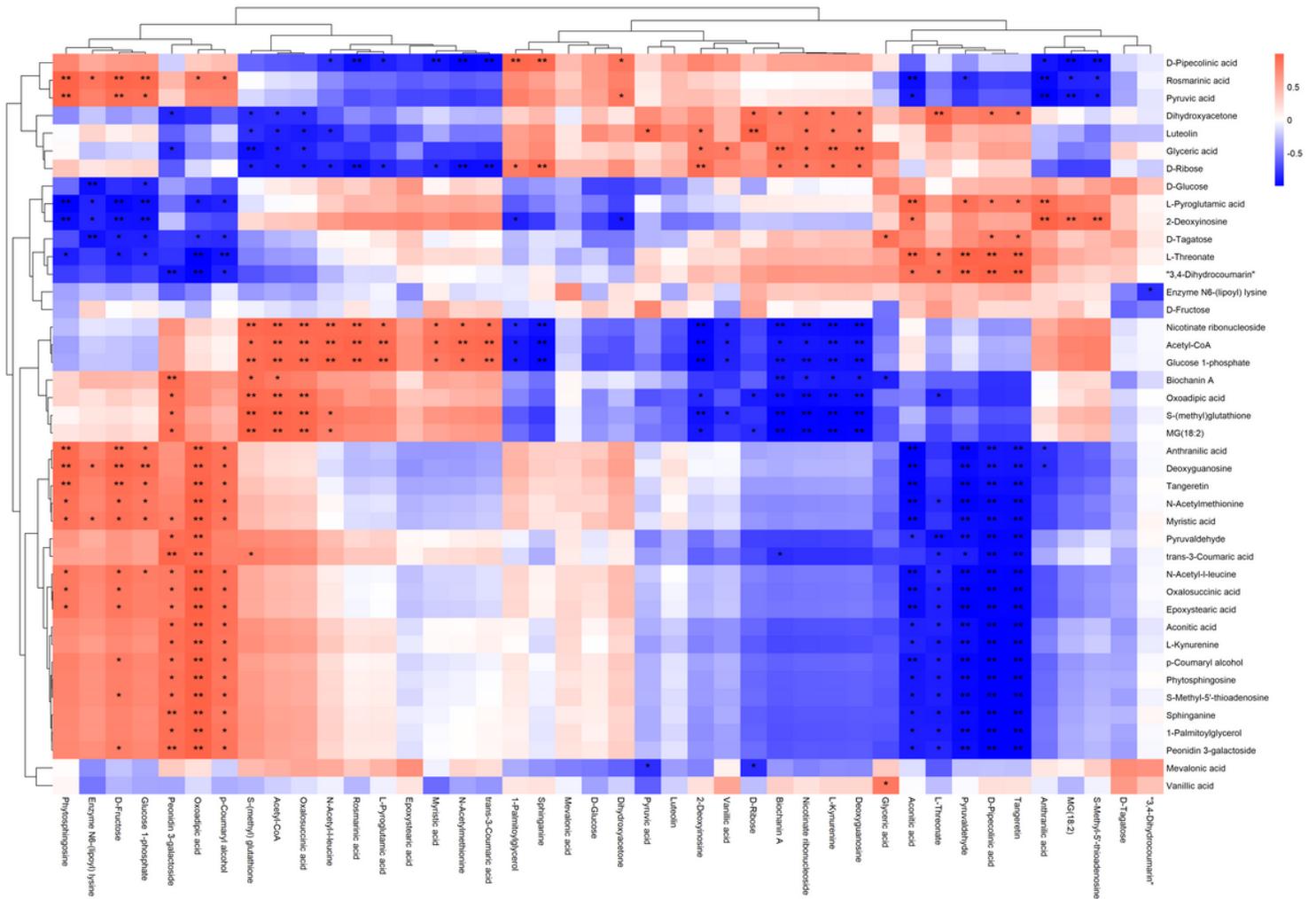


Figure 6

Correlation analysis of TS-DN3 leaf metabolites subjected to space flight treatment. A correlation matrix was generated using Pearson's correlation on six biological replicates of CK and SP-treated plants. Red colour shows positive correlation whereas blue colour represents negative correlation among the metabolites under space flight treatment. ** and * indicate significant difference at $p < 0.01$ and $p < 0.05$ by Pearson's correlation test, respectively.

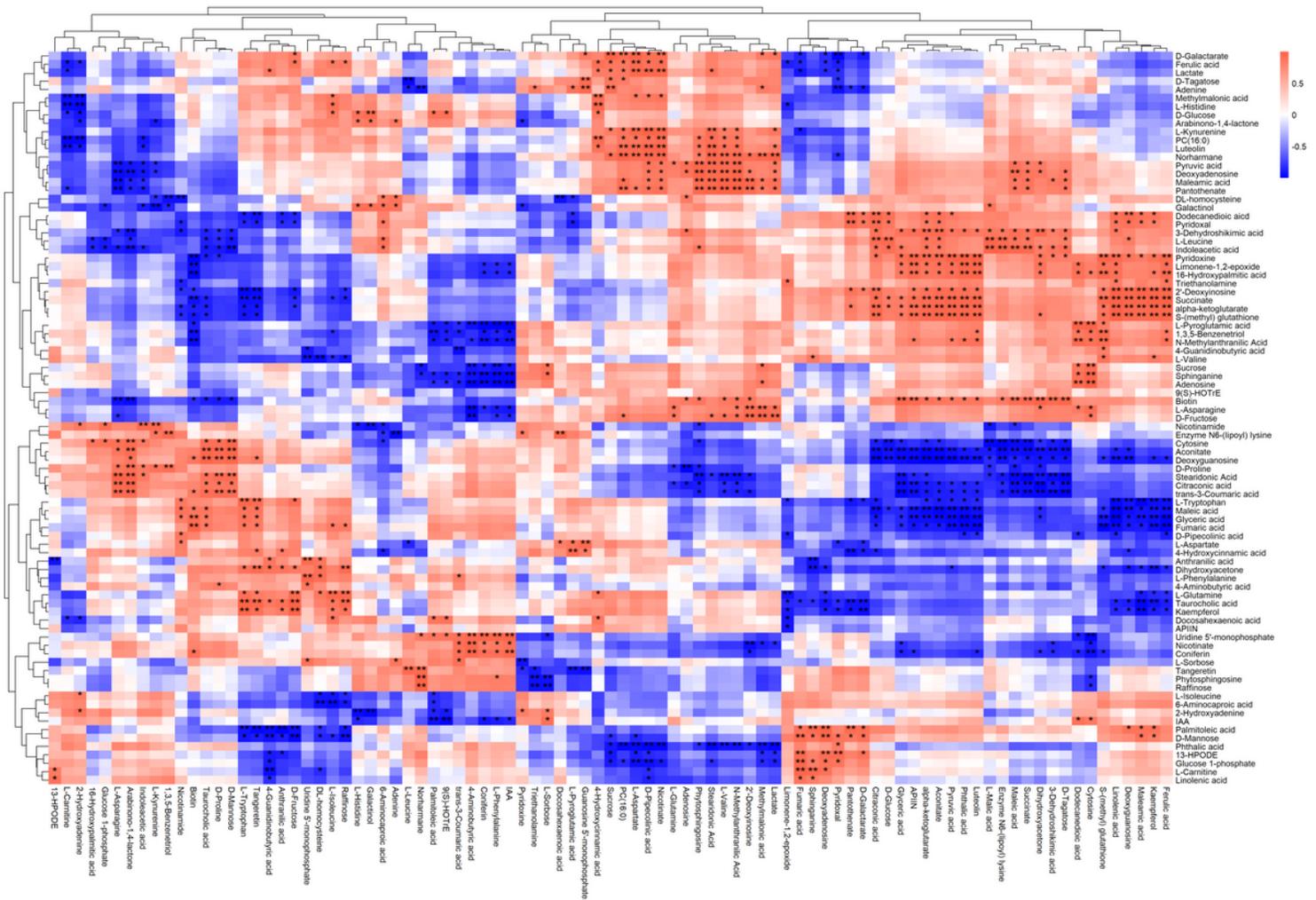


Figure 7

Correlation analysis of TLP-DN6 leaf metabolites subjected to space flight treatment. A correlation matrix was generated using Pearson's correlation on six biological replicates of CK and SP-treated plants. Red colour shows positive correlation whereas blue colour represents negative correlation among the metabolites under space flight treatment. ** and * indicate significant difference at $p < 0.01$ and $p < 0.05$ by Pearson's correlation test, respectively.

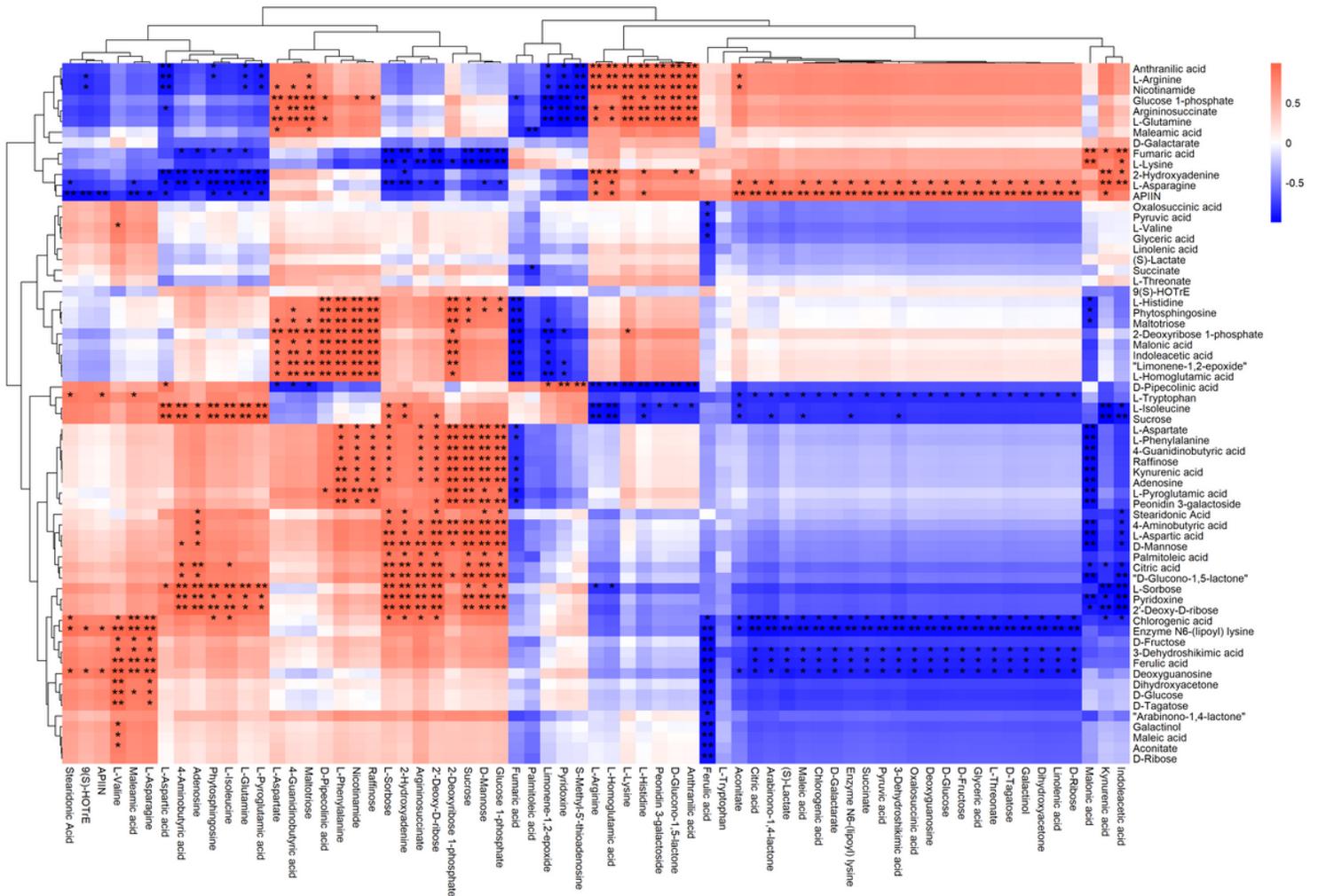


Figure 8

Correlation analysis of TS-DN6 leaf metabolites subjected to space flight treatment. A correlation matrix was generated using Pearson's correlation on six biological replicates of CK and SP-treated plants. Red colour shows positive correlation whereas blue colour represents negative correlation among the metabolites under space flight treatment. ** and * indicate significant difference at $p < 0.01$ and $p < 0.05$ by Pearson's correlation test, respectively.

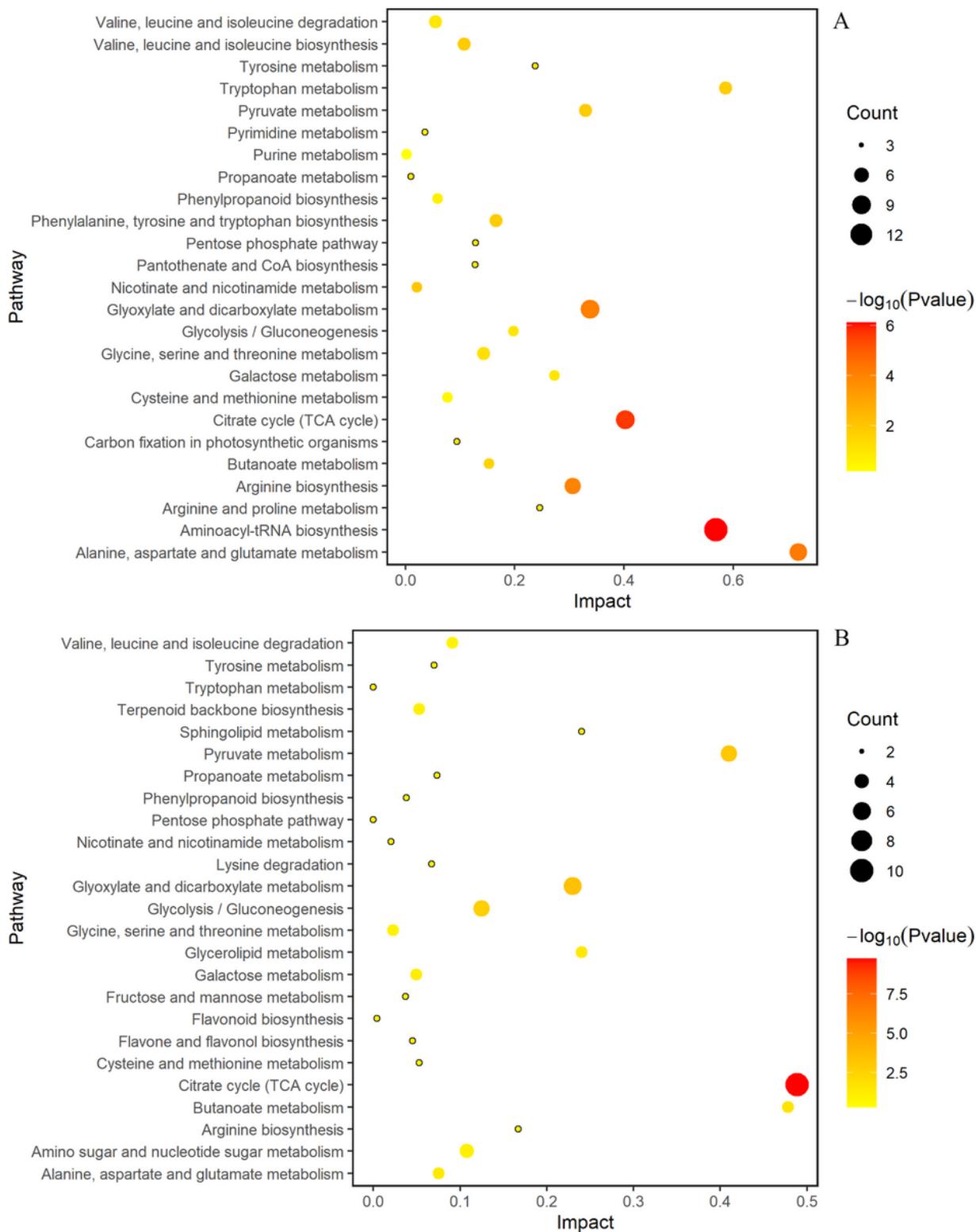


Figure 9

Analysis of metabolic pathways of TLP-DN3 and TS-DN3. Results were from the analyses using the MetaboAnalyst 4.0 software. Every circle represents a metabolic pathway, with red color indicating higher impact and yellow color indicating lower impact. The pathways with $p\text{-value} < 0.05$ were determined to have significant changes. The size of the circle represents the number of DEMs participating in the pathway. (A) Rice samples of DN3 at the TLP, (B) Rice samples of DN3 at the TS.

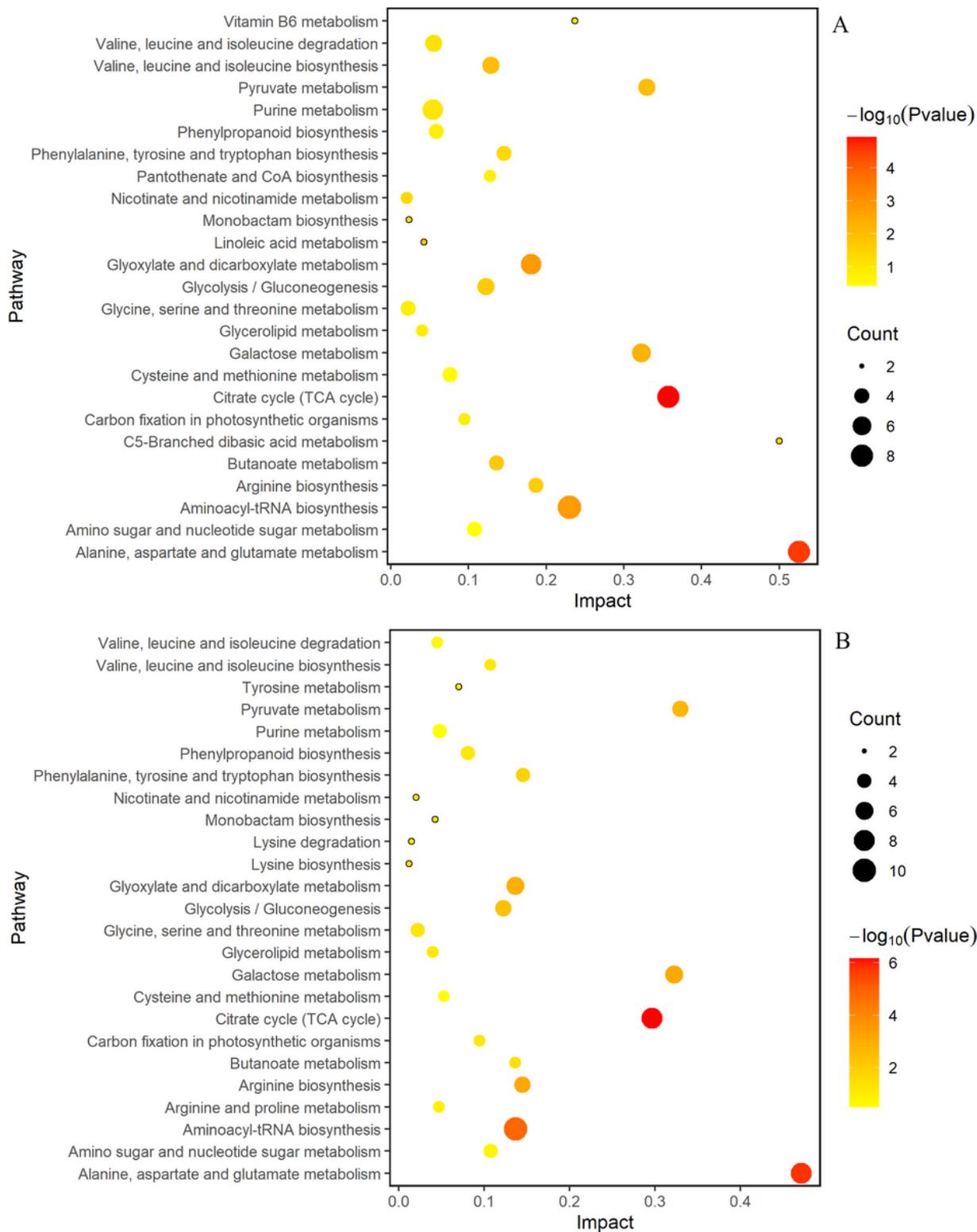


Figure 10

Analysis of metabolic pathways of TLP-DN6 and TS-DN6. Results were from the analyses using the MetaboAnalyst 4.0 software. Every circle represents a metabolic pathway, with red color indicating higher impact and yellow color indicating lower impact. The pathways with p-value < 0.05 were determined to have significant changes. The size of the circle represents the number of DEMs participating in the pathway. (A) Rice samples of DN6 at the TLP, (B) Rice samples of DN6 at the TS.

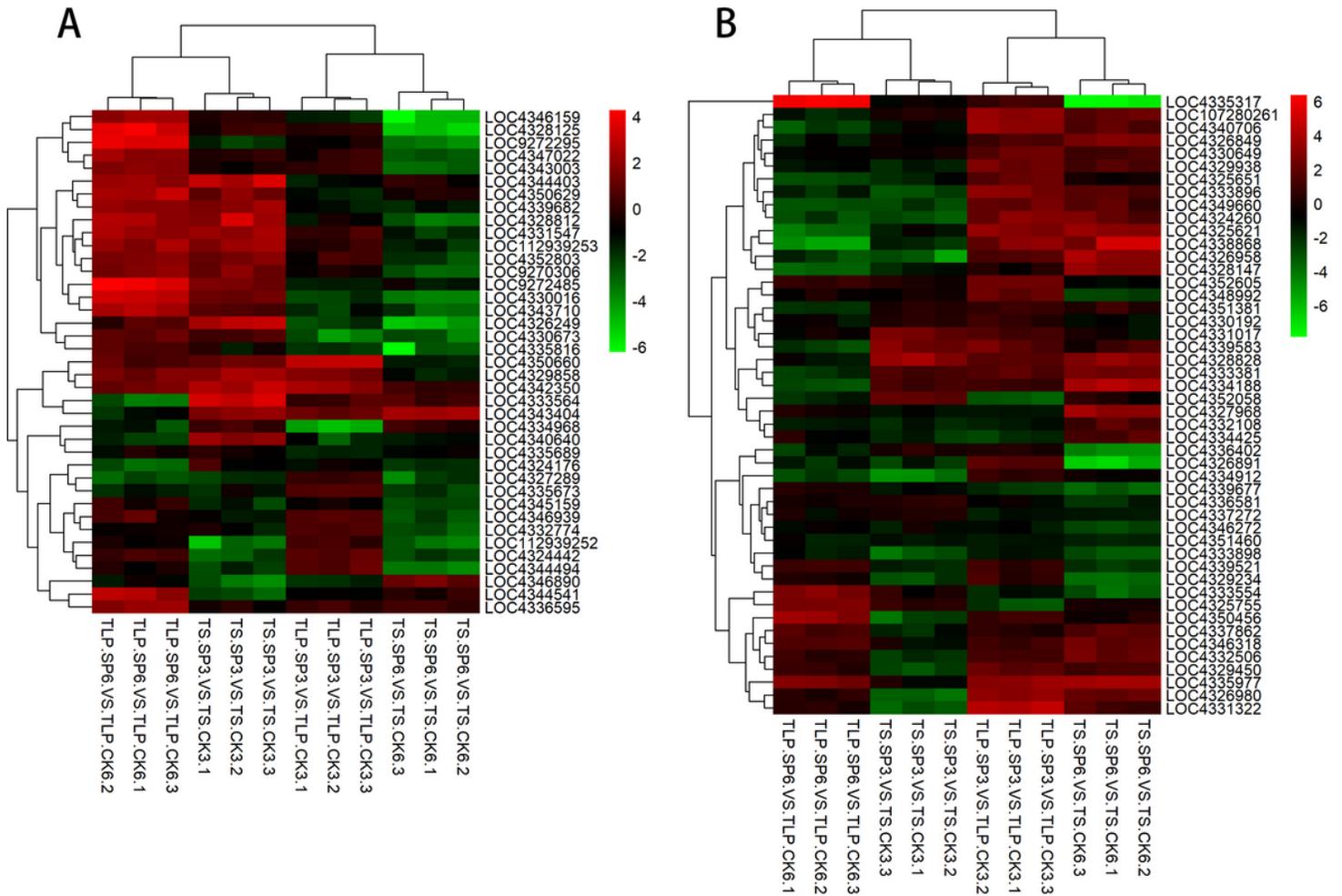


Figure 11

The relative expression levels of TCA cycle and amino acid metabolism genes in two different rice varieties. The heat map shows the expression fold change in rice leaves obtained by qRT-PCR. The relative expression is calculated by the gene expression of the space group and the control group at different growth periods. A: The expression of genes related to the TCA cycle. B: Expression of genes related to amino acid metabolism. Red indicates that the gene expression is up-regulated, and green metabolism is down-regulated. The normalization of gene expression was obtained by log₂ transformation. Hierarchical clustering analysis was performed using R.

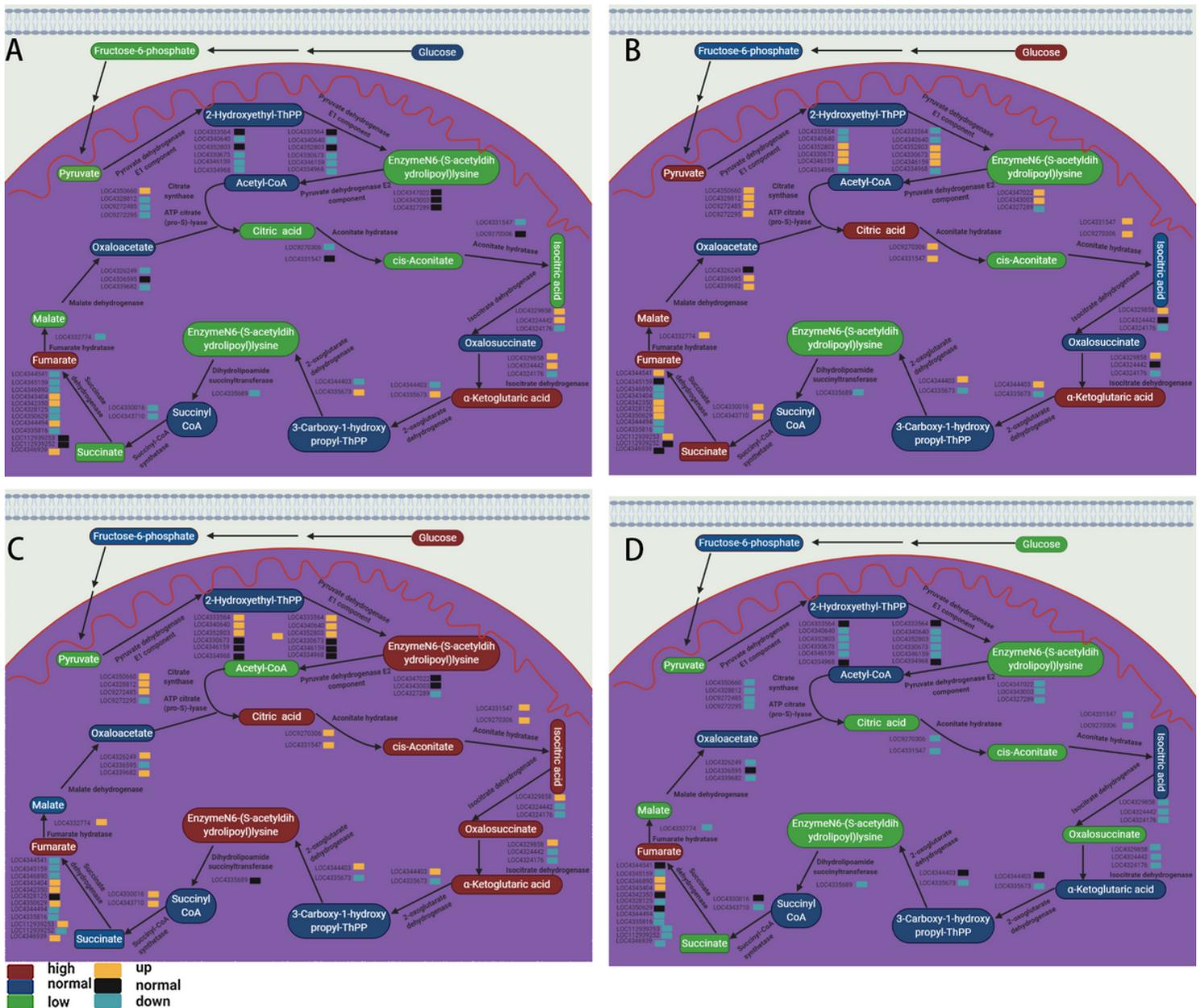


Figure 12

TCA cycle pathways of two rice varieties. After space flight, the two rice varieties developed into TLP and TS respectively. TLP-DN3 (A), TLP-DN6 (B), TS-DN3 (C), and TS-DN6 (D) show changes in the expression levels of metabolites and genes mapped to the TCA cycle pathway. The relative content of each metabolite in the grain is displayed in the form of a heat map from low (green) to high (red), as shown by the color scale. Enzymes related to these pathways are marked in red, and genes encoding enzymes are placed next to them. Similarly, gene expression levels are represented by yellow (up-regulated) and cyan (down-regulated). Blue and black respectively represent no significant changes in metabolites and gene expression.

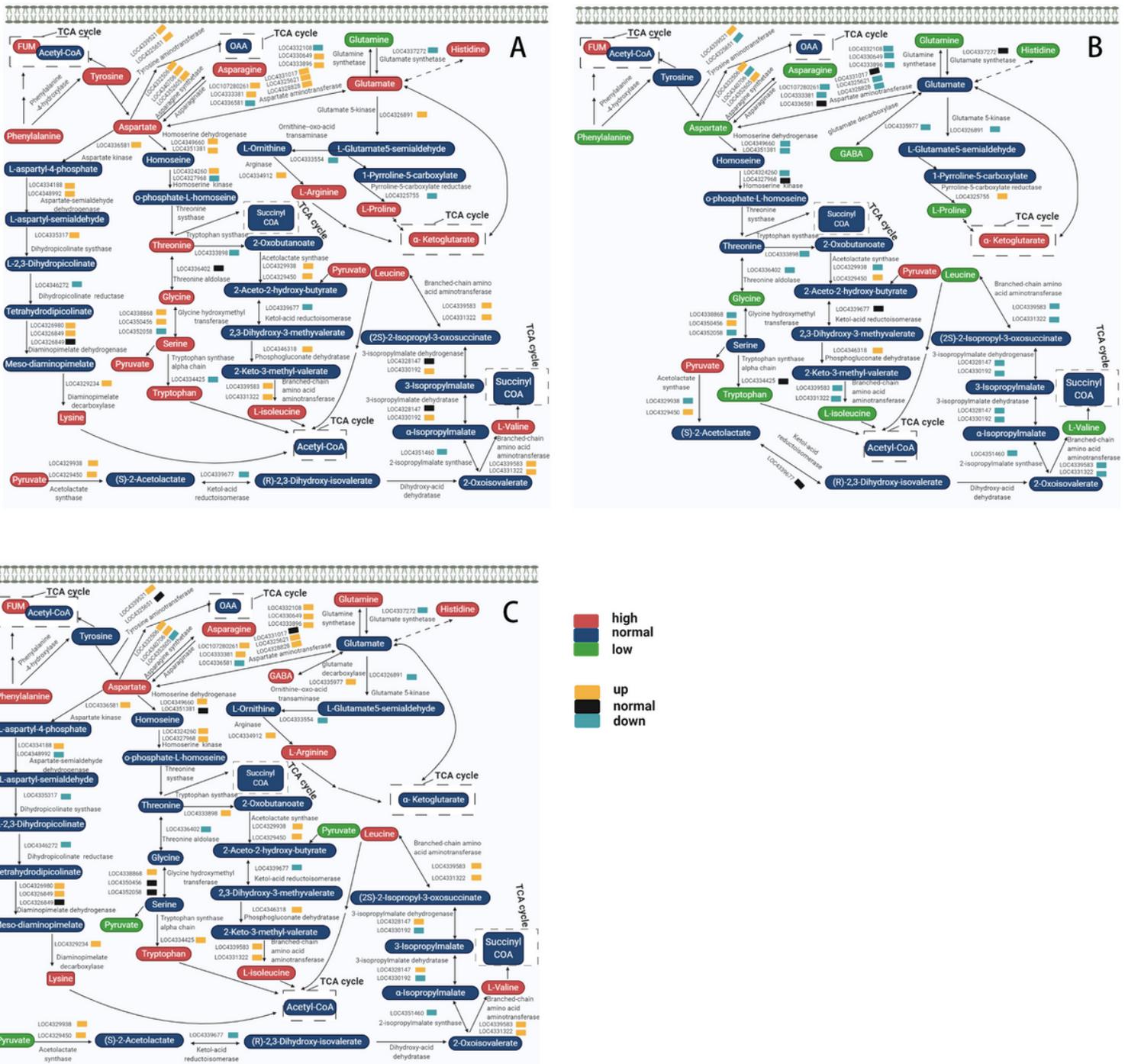


Figure 13

Amino acid metabolism pathways of two rice varieties. After space flight, the two rice varieties developed into TLP and TS respectively. TLP-DN3 (A), TLP-DN6 (B), and TS-DN6 (C) show changes in the expression levels of metabolites and genes mapped to amino acid metabolism pathways. The relative content of each metabolite in rice is displayed in the form of a heat map from low (green) to high (red), as shown by the color scale. Enzymes related to these pathways are marked in red, and genes encoding enzymes are placed next to them. Similarly, gene expression levels are represented by yellow (up-regulated) and cyan (down-regulated) respectively. Blue and black (respectively) represent no significant changes in metabolites and gene expression.

Supplementary Files

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

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