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Comparative Metabolomics Analysis of 3 New Potato Varieties against the Atlantic Cultivar; Bioinformatics based Experimental Study

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ABSTRACT

Background: Varietal differences in metabolite composition play a crucial role in determining agronomic traits, nutritional quality and stress resilience in *Solanum tuberosum*.

Methods: Three potatoes varieties; Longshu 7, Longshu 10 and Longshu 14 were selected and bred by the at the Potato Research Institute of Gansu Academy of Agricultural Sciences between December 2021 to December 2023. Thereafter, comparative metabolomics analysis of three new potato varieties (LS7, LS10 and LS14) was undertaken against the well-established Atlantic cultivar. We employed a bioinformatics-based experimental approach to analyze the metabolomic data obtained from the three new potato varieties (LS7, LS10 and LS14) and the Atlantic cultivar. Metabolite profiling was performed using high-throughput liquid chromatography-mass spectrometry (LC-MS) techniques. Differential metabolite analysis and pathway enrichment analysis were conducted to identify significant metabolic differences and enriched pathways among the varieties.

Result: Results revealed substantial differences in metabolite composition and pathway enrichment between the new potato varieties and the Atlantic cultivar. Metabolites involved in diverse metabolic pathways, including glucoside and hydroxyproline in LS7, kaempferol and ascorbic acid in LS10 and phenyl-butyryl-glutamine and ascorbic acid in LS14, exhibited significant upregulation or downregulation compared to the Atlantic cultivar. Pathway enrichment analysis highlighted the prominence of ABC transporters across all comparisons, suggesting their universal role in mediating cellular processes and stress responses in potato varieties. In conclusion, these findings contribute to our understanding of potato metabolism and pave the way for the development of novel breeding approaches to meet the evolving demands of global agriculture and food security.

Key words: Atlantic cultivar, Bioinformatics analysis, Comparative study, Metabolomics, Potato varieties.

INTRODUCTION

In China, the common potato varieties include Longshu 3, Favorita, Atlantic and Shepody, among others (Lee *et al*., 2021; Xiao *et al*., 2020). Longshu 3 is renowned for its high yield potential and adaptability to various soil types, making it popular among farmers across different regions. Favorita is prized for its disease resistance, particularly against late blight, a common threat to potato crops. Atlantic and Shepody varieties are favored for their excellent tuber quality, suitable for both fresh consumption and processing into value-added products. However, despite their respective strengths, these common potato varieties in China are not immune to certain weaknesses. Longshu 3, while high-yielding, may exhibit susceptibility to certain diseases under certain environmental conditions, requiring careful management practices. Favorita, despite its disease resistance, may have lower overall yield potential compared to other varieties. Atlantic and Shepody, although prized for their tuber quality, face challenges related to susceptibility to storage diseases and post-harvest handling issues. Addressing these weaknesses through targeted breeding efforts and agronomic strategies is necessary for sustaining and enhancing potato production in China (Huh *et al*., 1997; Lee *et al*., 2021; Machida-Hirano, 2015; Xiao *et al*., 2020).

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The utilization of bioinformatics approaches is essential for the interpretation of large-scale metabolomics data sets, facilitating the identification of key metabolites, metabolic pathways and regulatory networks in potatoes (Barupal *et al*., 2018). By integrating experimental findings

with computational analyses, we seek to unravel the complex metabolic mechanisms underlying the observed phenotypic variations among the potato varieties under investigation (Barben *et al*., 2010; Chourasia *et al*., 2021). There is need for more research that holds significant implications for potato breeding programs, agricultural sustainability, environmental resistant and human nutrition (Patel and Singh, 2021; Rabinovich and Fomicheva, 2018). In this context, the present research endeavors to conduct a comprehensive comparative metabolomics analysis of three newly developed potato varieties, in contrast to the well-established Atlantic cultivar. These novel varieties have been selected for their purported superior traits, including enhanced disease resistance, increased yield potential and improved nutritional content. Through a combination of experimental assays and bioinformatics tools, we aim to decipher the metabolic signatures that distinguish these varieties from the Atlantic cultivar.

MATERIALS AND METHODS

Description of potatoes varieties and cultivar

This study was undertaken at the Potato Research Institute of Gansu Academy of Agricultural Sciences, Gansu China. Three potatoes varieties; Longshu 7, Longshu 10 and Longshu 14 were selected and bred by the Potato Research Institute of Gansu Academy of Agricultural Sciences between December 2021 to December 2023. They were confirmed to be widely adaptable, stable and with high-yield in Xinjiang in the north and south of Xinjiang. The average yield per mu were 2278.01 kg, 4078.02 kg and 3278.02 kg respectively and the growth period to maturity were 109 days, 108 days and 105 days respectively. The three varieties were all late-maturing varieties and were found to be good for use in fresh food processing and use. The Longshu 7 had strong growth potential, with a maximum plant height of 155 cm, which could be controlled based on different soil fertility and field management level for improved the yield. Longshu 10 had strong adaptability, maximum plant height of 103 cm, number of blocks per plant were 3.8. Longshu 14 had stronger growth, strong adaptability and greater yield potential.

The selected Atlantic cultivar is a medium-ripening variety, with a growth period of 90 days, strong adaptability, plant height of 63.5 cm, reasonable nutrient distribution of aboveground and underground roots and tubers, with a yield of 3280.02 kg per mu, the number of main stems is 2.3, the nutrient supply is relatively concentrated, the number of blocks per plant is 5.1, the commercial potato rate is high and with good stable yield. This variety needs fine management, likes water and fertilizer conditions of good plots. It has the characteristics of high starch content and low reducing sugar content. It is the preferred variety by many potato seed enterprises and is more welcomed and favored by the majority of processing enterprises in Xinjiang.

Experimental design

The whole project testing process includes sample preparation, QC sample preparation, sample LC-MS/MS mass spectrometry analysis and data quality and control and data analysis. In this study, ultra-performance liquid chromatography-tandem electrostatic field orbitrap mass spectrometry (UHPLC-Q Exactive HFX) was used. Metabolites in samples were detected by comparing the retention time and molecule of metabolites in local and commercial databases. Mass (metabolomics) molecular mass error within <10 ppm), secondary fragmentation spectra and other information were matched to metabolize the biological samples. Qualitative and quantitative detection and subsequent data analysis.

Sample extraction

Solid samples (Plants)

After the samples were slowly thawed at 4°C, appropriate amount of sample (50-100 mg) was taken and added to 1ml of a mixture of water, acetonitrile and isopropanol at a ratio of 1:1:1, v/v). The mixture was vortexed for 60s, subjected to cryogenic sonication for 30 min, centrifuged at 12000 rpm at 4°C for 10 min and the supernatant was obtained and placed at -20°C. After 1 hour, the precipitated protein was obtained centrifuged for at 12000 rpm at 4°C for 10 min. The supernatant was obtained vacuum dried and reconstituted in 200 ul 30% CAN and vortexed and centrifuged at 14000 rpm at 4°C for 15 min and the supernatant obtained.

Liquid samples

0.5-1 ml of the appropriate volume of sample was taken and when the sample volume was too large, freeze-drying was done for concentration. 2 times the volume of methanol and acetonitrile solution of 1:1, v/v) was added to the extract, vortexed for 60s. Thereafter subjected to low temperature ultrasonication for 30 min, centrifuged at 12000 rpm at 4°C for 10 min. The supernatant was then placed at -20°C for 1 h to precipitate proteins. The obtained protein was the centrifuged at 12000 rpm at 4°C for 10min and the 100 μl supernatant was obtained and freeze-dried and reconstituted in 100 μl 30% CAN. It was then vortexed and centrifuged at 12000 rpm at 4°C for 10 min and the supernatant was detected on the machine.

Liquid chromatography parameters

Mobile phase

Phase A consisted of a 0.1% formic acid-aqueous solution, while phase B comprised a 0.1% formic acid-acetonitrile solution.

Flow rate

The flow rate was set at 0.3 ml/min.

Column temperature

The column temperature was maintained at 40°C throughout the analysis.

Injection volume

A volume of 2 µl was injected for analysis.

Elution gradient

The elution gradient started with phase B maintained at 0% from 0.0 to 1.0 minutes, followed by a linear increase from 0% to 95% from 1.0 to 12.0 minutes, holding at 95% B from 12.0 to 13.0 minutes, linearly decreasing from 95% to 0% from 13.0 to 13.1 minutes and finally, maintaining at 0% from 13.1 to 17.0 minutes.

Sample handling

Samples were stored in a 4°C autosampler to maintain stability. A random sequence was employed during analysis to mitigate instrument signal fluctuations. Quality control (QC) samples were intermittently analyzed to ensure system stability and experiment reliability.

Mass spectrometry conditions

The primary and secondary spectra were obtained using the Q Exactive HFX high-resolution mass spectrometry system manufactured by Thermo Company. Electrospray ionization (ESI) was employed under the following conditions: sheath gas at 40 arb; auxiliary gas at 10 arb; spray voltage set at 3000 V/-2800V; temperature maintained at 350°C and ion transfer tube temperature at 320°C. The scanning mode utilized was Full-ms-ddMS2 mode, with scanning conducted in both positive and negative modes. For Level 1 scans in the metabolomics analysis, the mass-to-charge ratio (m/z) range was set from 70 to 1050 Da, with a resolution of 70000, while for Level 2 scans, the resolution was adjusted to 17500.

Data preprocessing

The raw data underwent processing utilizing the Progenesis QI metabolomics software developed by Waters Corporation, based in Milford, USA.

Principal component analysis (PCA) analysis

Principal component analysis (PCA) was used to aggregates all identified metabolites into a new linear combination, generating a set of comprehensive variables. From this set, several variables were selected to encapsulate as much information as possible from the original dataset, facilitating dimensionality reduction.

Partial least squares discriminant analysis (PLS-DA) analysis

PLS-DA was used to categorize each component, facilitating the identification of similarities and differences between different groups. In cases where the differences between groups were not sufficiently pronounced for PCA to effectively differentiate them, PLS-DA was applied for the enhanced efficacy. During PLS-DA analysis, data normalization was achieved using Pareto scaling.

Orthogonal partial least squares discriminant analysis (OPLS-DA) analysis

Orthogonal partial least squares discriminant analysis (OPLS-DA) which is a derivative algorithm stemming from PLS-DA was used to model the relationship between metabolite expression and sample class, enabling sample type prediction. In this study, data normalization using Pareto scaling was conducted for OPLS-DA analysis. Model evaluation parameters, such as R2Y and Q2, are assessed, where a Q2 value exceeding 0.5 indicates model stability and reliability. Q2 values falling between 0.3 and 0.5 signify good model stability, while Q2 values below 0.3 suggest lower model reliability.

Kyoto encyclopedia of genes and genomes (KEGG)

By integrating information from the Kyoto Encyclopedia of Genes and Genomes (KEGG) compound database for differential metabolites obtained, the pathways of the differential metabolites were then annotated and divided by the KEGG database to obtain metabolic pathway maps of differential metabolites.

Results and Discussion

Heatmap

Valuable insights for crop improvement strategies is necessary in enhancing resilience, nutritional quality, and overall productivity of potatoes as had been recommended

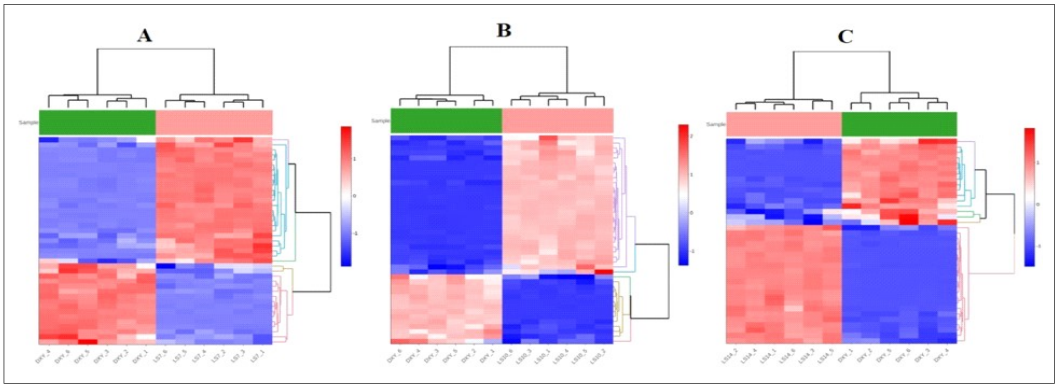


Fig 1: Heat maps of metabolites between DXY vs LS7 (A), DXY vs LS10 (B) and DXY vs LS14 (C).

in other studies (Kahar *et al*., 2017; Yimer, 2022) . Heatmap Detected metabolites were represented in heat maps indicating the number of metabolites between the 3 new potato varieties and the Atlantic cultivar (Fig 1). Based on this result, it was shown that more metabolites were detected in the 3 new varieties (DS7, DS10 and DS14) than in the Atlantic cultivar (DXY).

Volcano plot

Univariate analysis stands were applied to identify differential metabolites between two groups of samples, which applied fold change (FC) and the T-test to generate volcano plots. This enabled the visual representation of metabolite changes between the 3 new potato varieties and the Atlantic cultivar, aiding in the identification of

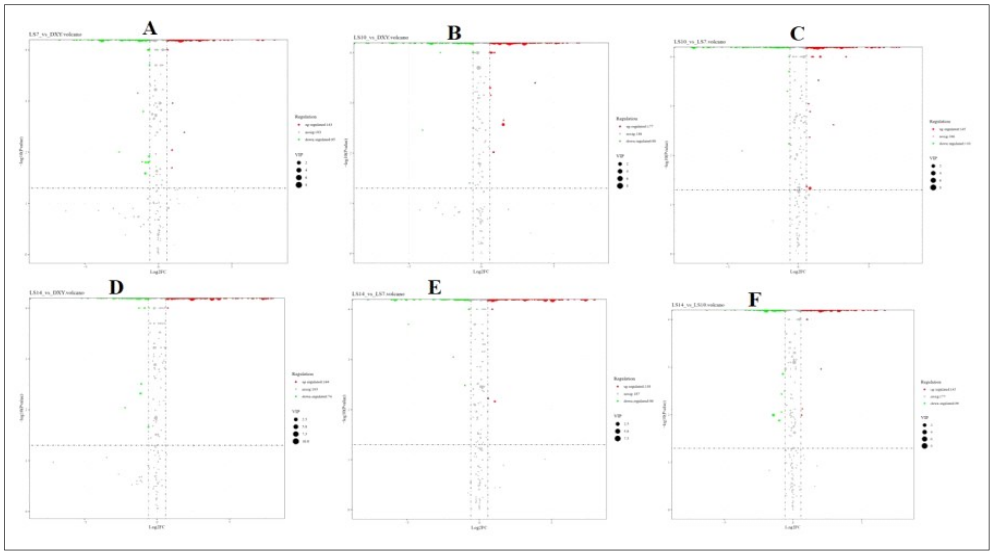


Fig 2: Box plots of upregulated and down regulated defferential metabolites between LS7 vs DXY (A), LS10 vs DXY (B), LS10 vs LS7 (C), LS14 vs DXY (D), LS14 vs LS7 (E) and LS14 vs LS10 (F).

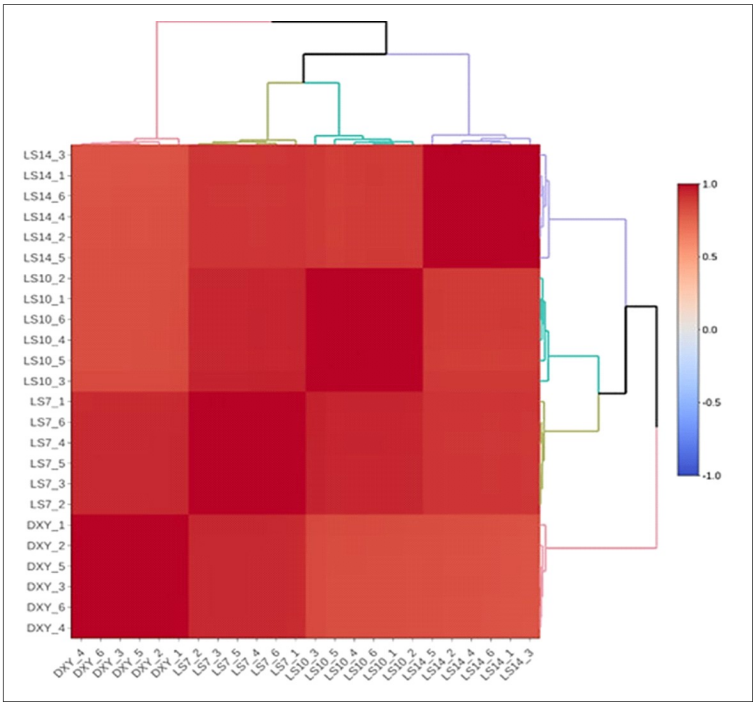


Fig 3: Interrelationship changes between the 3 new potato varieties and the atlantic cultivar through heatmap.

potential marker metabolites. The differential metabolites identified were the displayed in volcano plot (Fig 2). In these plots, the criteria for screening differential metabolites were set as FC > 1.5 or FC < -1.5, along with a p-value less than 0.05. Thus, the differential metabolites highlighted in the volcano plot are those identified through univariate statistical analysis. Based on that, it was shown that there were more upregulated metabolites than downregulated metabolites across all the comparative groups [LS7 (143) verses DSY (95), LS10 (177) verses DXY (98), LS10 (145) verses LS7 (110), IS14 (164) verses DXY (74), LS14 (138) verses LS7 (96) and LS14 (145) verses LS10 (95)].

Correlation analysis of differential metabolites

Correlation analysis was applied to assess the extent of correlation among significant differential metabolites. The result provided in Fig 3 indicated that there was high significant correlation in metabolites between the 3 new varieties (DS7, DS10 and DS14), pointing to high changes and differences in metabolites between the 3 varieties from the Atlantic cultivar.

ChemRICH enrichment analysis chart

Based Chemical Similarity Enrichment Analysis (ChemRICH), it was noted that glucoside and hydroxyproline were respectively the most upregulated and down regulated metabolites under LS7 verses DXY groups, kaempferol and ascorbic acid were respectively the most upregulated and down regulated metabolites under LS10 verses DXY groups, caffeoilglucopyranose and trymethylammonobutanoic acid were respectively the most upregulated and down regulated metabolites under LS10 verses LS10 groups, phenyl-butyryl-glutamine and ascorbic acid were respectively the most upregulated and down regulated metabolites under LS14 verses DXY groups, feruloyl lysine and trymethylammonobutanoic acid were respectively the most upregulated and down regulated metabolites under LS14 verses LS7 groups and feruloyl lysine and glucoside were respectively the most upregulated and down regulated metabolites under LS14 verses LS10 groups. The proportion of the number of metabolites were presented as a pie chart which showed that organooxygen compounds were the most dominant metabolites under LS7 verses DXY group (Fig 4a), carboxylic acid and derivatives were the most dominant metabolites under both LS10 verses DXY (Fig 4b) and LS14 verses DXY (Fig 4c) groups.

Metabolic pathway analysis

The identified differential metabolites were obtained and subsequently, the pathways associated with these differential metabolites were annotated and categorized using the KEGG database to generate metabolic pathway maps. These metabolites were analyzed and computed using Fisher's exact test to determine the level of enrichment significance to aid in identifying metabolic and signal transduction pathways that are notably affected. It was shown that taste transduction, glutathione metabolism, ABC transporters, biosynthesis of amino acid, central carbon metabolism in cancer, arginine and proline

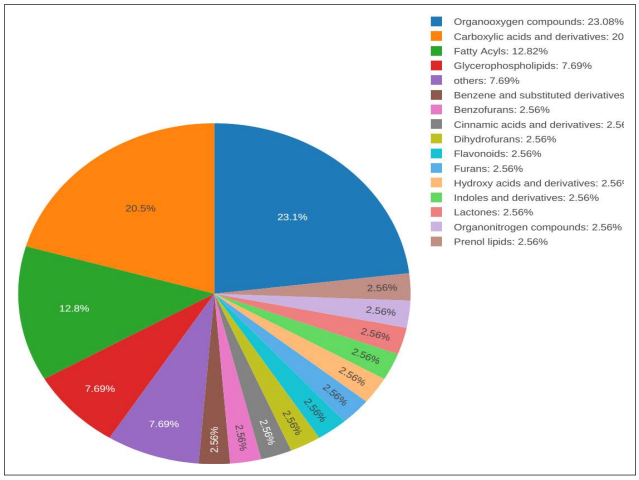


Fig 4a: Dominant metabolites under LS7 verses DYX group.

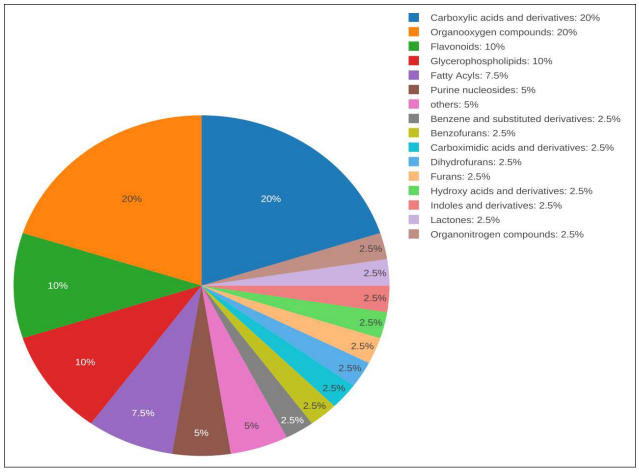


Fig 4b: Dominant metabolites under LS10 verses DXY group.

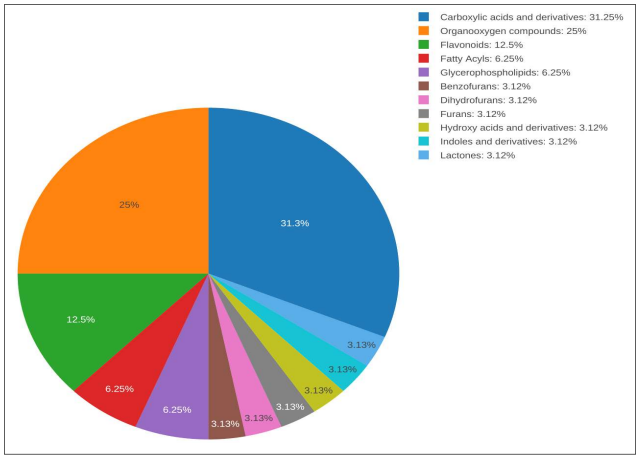


Fig 4c: Dominant metabolites under LS14 verses DXY group.

metabolism and alanine, aspartate and glutamate metabolism were the most enriched pathways between LS7 verses DXY groups (Fig 5a). ABC transporters was the most enriched pathway between LS10 verses DXY groups (Fig 5b). Glutathione metabolism and ABC transporters were the most enriched pathway between LS10 verses DXY groups (Fig 5c).

The differential abundance score (DAS) which a metric that indicates the difference in abundance between upregulated and downregulated metabolites annotated to a specific pathway was used to represents the number of differential metabolites enrichment associated with the pathway. This was to effectively capture the average and overall changes occurring across all metabolites within a

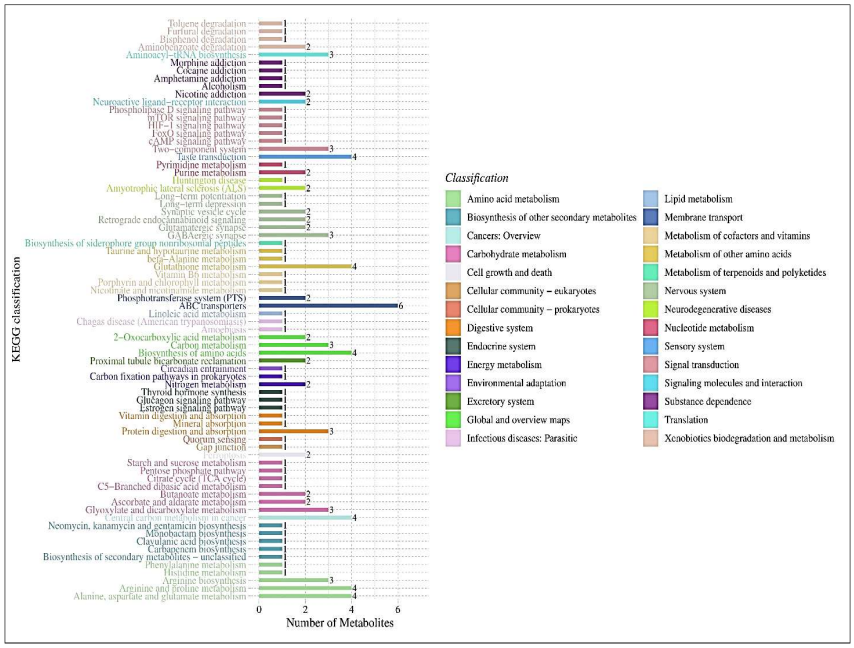


Fig 5a: KEGG annotation between LS7 Vs DXY.

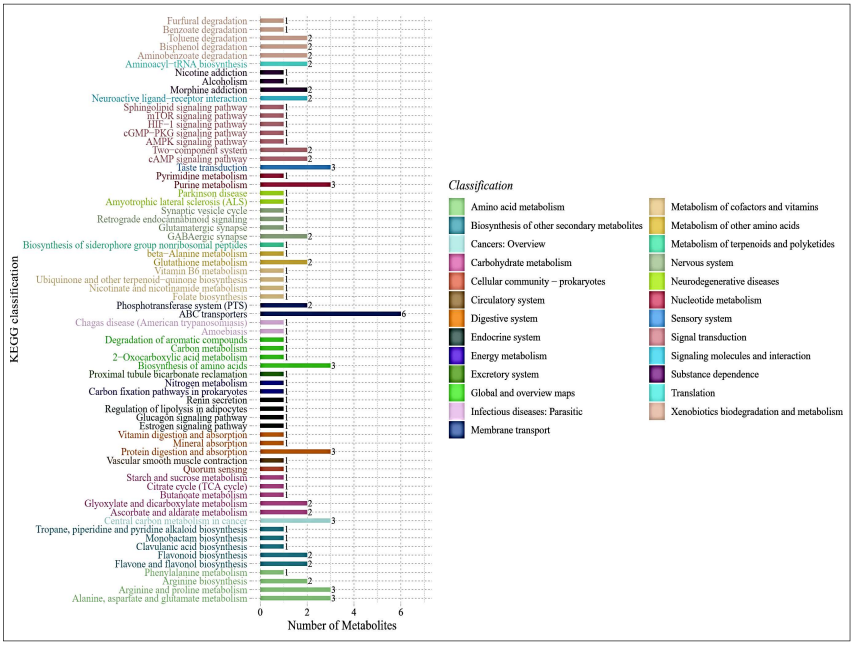


Fig 5b: KEGG annotation between LS10 VS DXY.

pathway (Fig 6a, 6b and 6c). Based on this analysis, it was indicated that ABC transporters was the most enriched pathway with significant Fisher test under LS7 verses DXY group, LS10 verses DXY group as well as under LS14 verses DXY groups.

To facilitate comparison between different pathways, the composite score for each pathway is normalized to 1. Biomolecules are assigned weighted scores based on their relative positional importance within the pathway. By calculating the weighted score of matched metabolisms, a cumulative importance score for the pathway was obtained. A higher score indicated a greater impact of the pathway. Within the LS7 verses DXY groups, protein digestion and adsorption was the most dominant metabolic

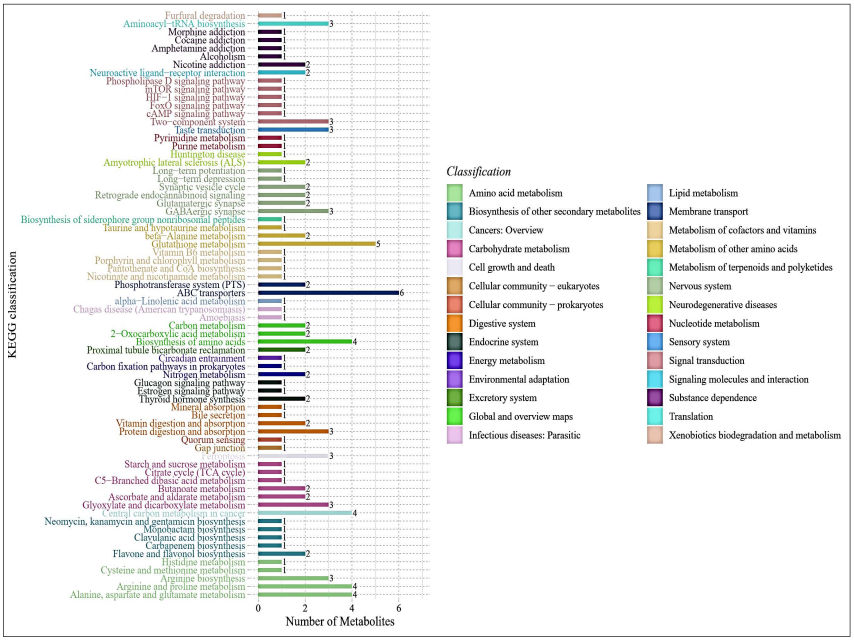


Fig 5c: KEGG annotation between LS14 VS DXY.

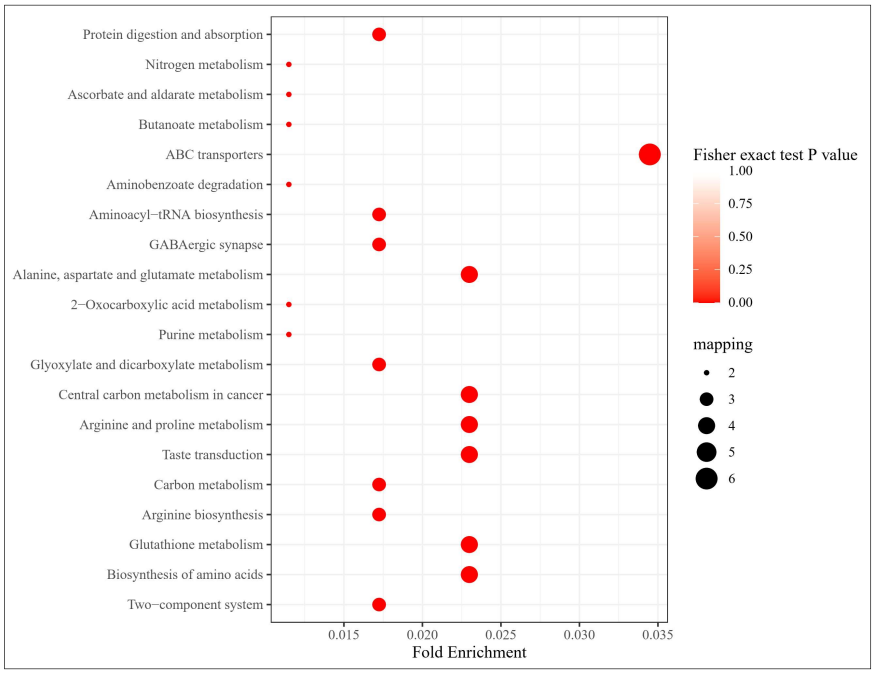


Fig 6a: KEGG enrichment analysis between LS7 VS DXY.

process under the organismal systems process pathway category, nitrogen metabolism was the most significantly dominant metabolic process under the metabolism pathway category, nicotine addiction was the most significantly dominant metabolic process under the human diseases process pathway category, while under environmental information process pathway, phospholipase-D-signaling pathway and FoxO signaling pathway and cAMP siganaling pathway had no significant difference in dominance (Fig 7a). Within the LS10 verses DXY groups, protein digestion and adsorption was the most dominant metabolic process under the organismal systems process pathway category, bisphenol degradation and ascorbic and aldarate metabolism were the most significantly dominant metabolic process under the metabolism pathway category, morphine addiction was the most significantly

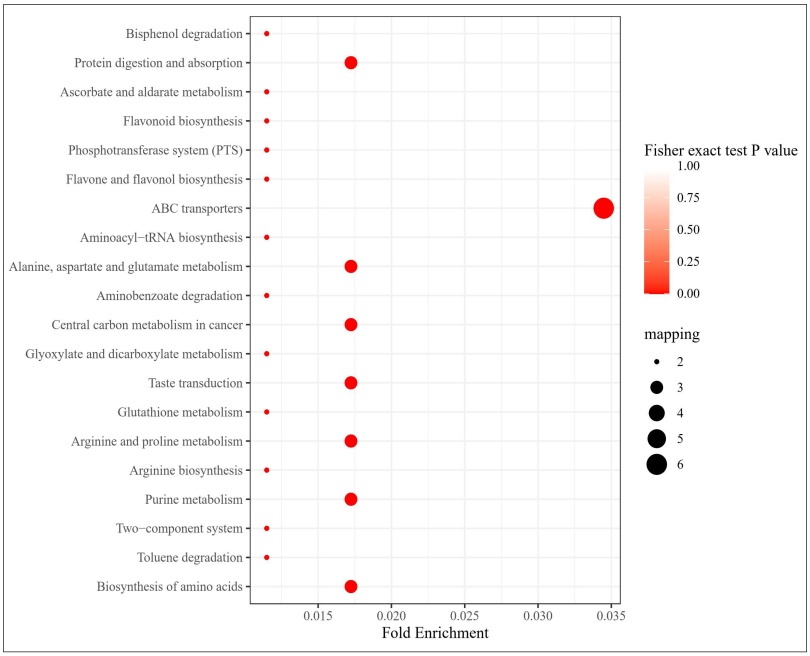


Fig 6b: KEGG enrichment analysis between LS10 VS DXY.

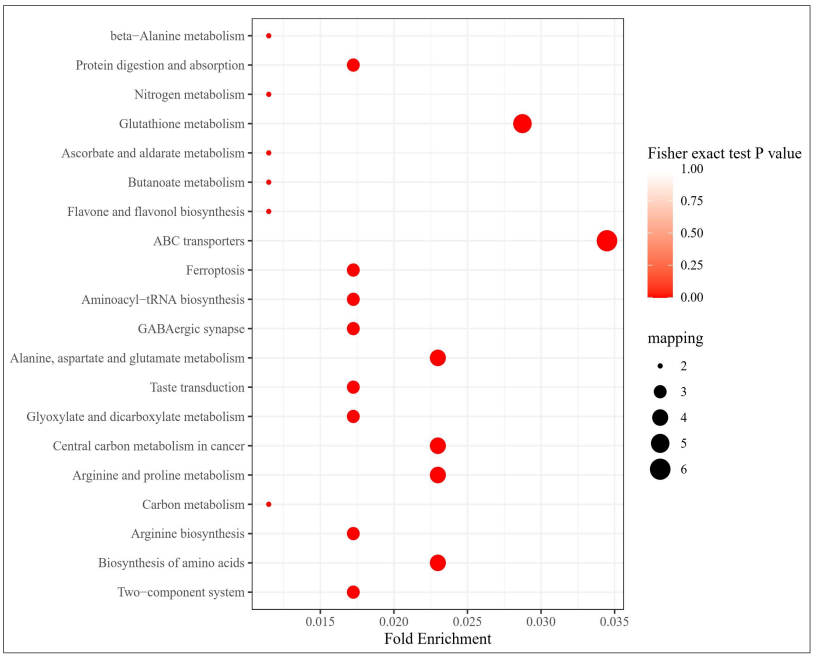


Fig 6c: KEGG enrichment analysis between LS14 VS DXY.

dominant metabolic process under the human diseases process pathway and cAMP siganaling pathway was the most significantly dominant metabolic process under environmental information process pathway (Fig 7b). Within the LS14 verses DXY groups, protein digestion and adsorption was the most dominant metabolic process under the organismal systems process pathway category, nitrogen metabolism was the most significantly dominant metabolic process under the metabolism pathway category, nicotine addiction was the most significantly dominant metabolic process under the human diseases process pathway category, while under

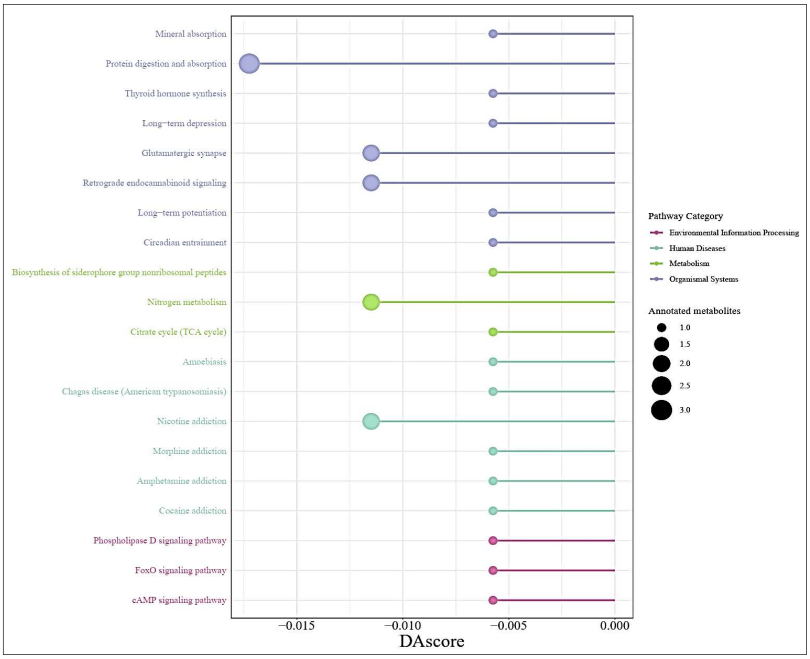


Fig 7a: Pathway categories through differential abundance (DA) scores between LS7 VS DXY.

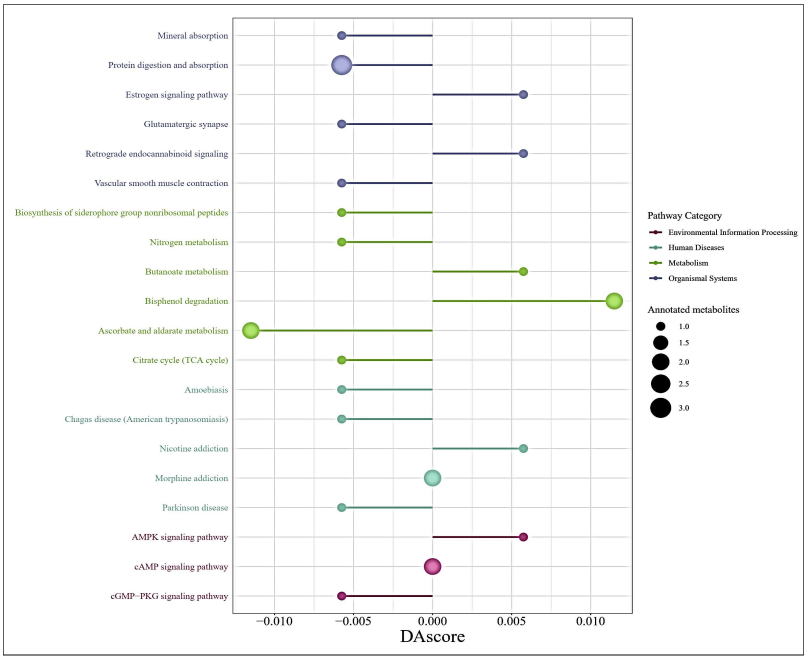


Fig 7b: Pathway categories through differential abundance (DA) scores between LS10 VS DXY.

environmental information process pathway, phospholipase-D-signaling pathway and FoxO signaling pathway and cAMP siganaling pathway had no significant difference in dominance (Fig 7c).

Detection of more metabolites in the three new varieties (DS7, DS10 and DS14) compared to the Atlantic cultivar (DXY) suggest a potentially richer metabolic profile in these new varieties. This finding could signify enhanced biochemical diversity and metabolic activity in the newer cultivars, possibly due to genetic variations or environmental influences (Martínez-García *et al*., 2001; Odgerel and Bánfalvi, 2021). These findings also underscore the importance of assessing metabolomic profiles to understand the biochemical intricacies underlying varietal differences and may inform breeding programs aiming to enhance crop resilience and quality (Lekota *et al*., 2020; Martínez-García *et al*., 2001; Odgerel and Bánfalvi, 2021). The high correlation observed among significant differential metabolites within the three new varieties (DS7, DS10 and DS14) compared to their correlation with the Atlantic cultivar (DXY) signifies pronounced metabolic distinctions between the new varieties and the established cultivar. This may suggest substantial metabolic reprogramming or divergence in the newly developed varieties, potentially reflecting unique genetic backgrounds or selective breeding efforts aimed at specific traits. This outcome contrasts with previous studies where a closer correlation was observed between varieties within the same cultivar, indicating a higher degree of metabolic similarity (Lekota *et al*., 2020; Tan *et al*., 2024).

The detailed analysis of differential metabolites between the experimental groups reveals specific metabolic signatures associated with each comparison, providing insights into the biochemical responses underlying varietal differences. Glucoside and hydroxyproline emerged as the most upregulated and downregulated metabolites, respectively, in the LS7 versus DXY comparison. Similarly, kaempferol and ascorbic acid were respectively identified as the most upregulated and downregulated metabolites in the LS10 versus DXY comparison, highlighting potential roles in antioxidant defense and redox homeostasis (Singh, 2018; Tan *et al*., 2024). Notably, the LS10 versus LS10 comparison revealed caffeoilglucopyranose and trimethylammonobutanoic acid as the most upregulated and downregulated metabolites, indicating intravarietal metabolic fluctuations possibly influenced by environmental factors or developmental stages (Oertel *et al*., 2017; Tan *et al*., 2024). In contrast, the comparisons involving the LS14 variety unveiled distinct metabolic alterations, with phenyl-butyryl-glutamine and ascorbic acid emerging as the most upregulated and downregulated metabolites in the LS14 versus DXY comparison. This suggests unique metabolic adaptations in LS14, potentially associated with stress responses or developmental processes. Furthermore, feruloyl lysine and trimethylammonobutanoic acid were respectively highlighted as the most upregulated and downregulated metabolites in the LS14 versus LS7 comparison, while feruloyl lysine and glucoside exhibited similar trends in the LS14 versus LS10 comparison (Barben *et al*., 2010; Odgerel and Bánfalvi, 2021; White *et al*., 2018).

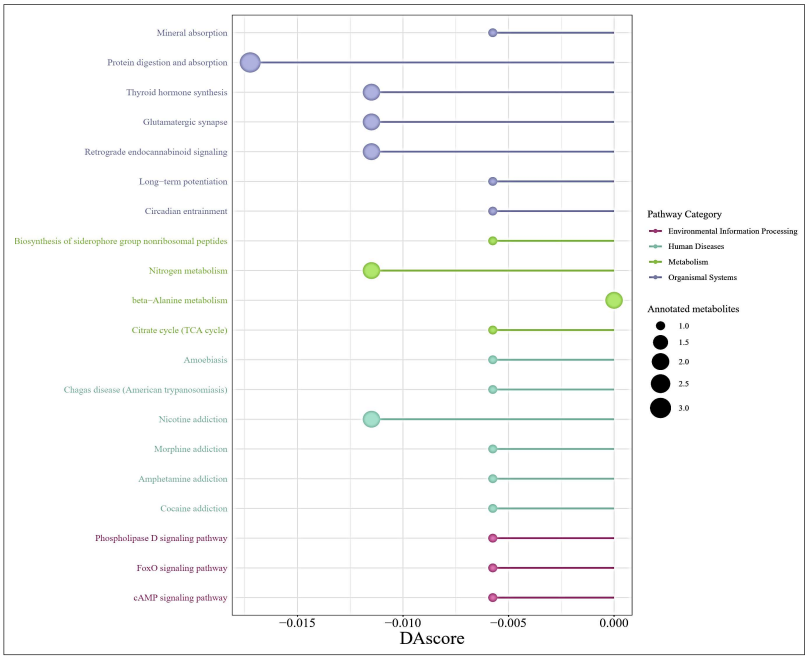


Fig 7c: Pathway categories through differential abundance (DA) scores between LS14 VS DXY.

The enrichment analysis of pathways between the LS7 and DXY groups revealed several significantly enriched pathways, including Taste transduction, glutathione metabolism, ABC transporters, biosynthesis of amino acids, central carbon metabolism in cancer, arginine and proline metabolism and alanine, aspartate and glutamate metabolism. The analysis revealing ABC transporters as the most enriched pathway across all comparisons LS7 versus DXY, LS10 versus DXY and LS14 versus DXY suggests a fundamental role for these transporters in mediating cellular processes and responses to environmental stimuli within the studied varieties. The consistent enrichment of this pathway across different varieties suggests its universal importance in plant metabolism and stress responses, emphasizing its potential as a target for crop improvement strategies aimed at enhancing nutrient uptake efficiency, stress tolerance and overall productivity (Barupal *et al*., 2018; Chea *et al*., 2021; Lekota *et al*., 2020).

Nitrogen metabolism exhibited significant dominance under the metabolism pathway category in all comparisons, indicating a conserved role in nitrogen utilization and assimilation among the studied varieties. Interestingly, while nicotine addiction was the most dominant process under the human diseases pathway category in all comparisons, the environmental information pathway category showed variable dominance, with some pathways exhibiting no significant difference, such as phospholipase-D-signaling pathway, FoxO signaling pathway and cAMP signaling pathway. However, the LS10 versus DXY comparison highlighted cAMP signaling pathway as the most significantly dominant process, suggesting potential varietal-specific responses to environmental cues mediated by cyclic AMP signaling. These findings contribute to our understanding of the metabolic landscape in the studied varieties and provide insights into potential pathways underlying varietal differences in physiological processes, stress responses and environmental interactions (Haas *et al*., 2009; Oertel *et al*., 2017; Xiao *et al*., 2020).

Conclusion

These results not only advance our understanding of plant metabolism and its regulation but also provide valuable insights for crop improvement strategies aimed at enhancing resilience, nutritional quality and overall productivity of potatoes as had been recommended in other studies. Further investigations into the functional significance of identified pathways and metabolites are warranted to elucidate their roles in plant physiology and their potential applications in agriculture and biotechnology.

Conflict of interest

The researchers affirm that there are no conflicts of interest associated with this study.

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