Metabolomics Profiling of Brassinolide and Abscisic Acid In Response to High Temperature Stress

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Research Article

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Abstract

To determine the effect of abscisic acid (ABA), brassinolide (BR) and ABA+BR on grape quality under high temperature stress (HTS), various metabolites were analyzed. Compared with the control (CK), DL-tryptophan, D-raffinose, geniposidic acid, dodecanedioic acid and polyphenols were found to be higher after ABA treatment. After BR treatment, amino acids and poricoic acid B were higher than in CK. And carbohydrates and amino acids were upregulated after ABA+BR treatment. BR and ABA+BR treatment also induced higher endogenous ABA and epibrassinolide (EBR) contents. In addition, treated grape had higher soluble solid concentrations (SSC) and soluble sugar content, and delayed the degradation of middle lamella and microfibrils. Antioxidant and heat shock related genes were examined, which significantly increased in treated grape. The finding of this study suggested that ABA, BR and ABA+BR are very useful for alleviating high temperature damage by increasing the accumulation of osmotic adjustment substances, and endogenous hormones content.

Key Message

Hormone treatment enhanced the content of osmotic substances under high temperature conditions. The effect of ABA and BR treated separately is better than treated together.

Introduction

Grape (*Vitis vinifera L.*) is one of the most popular horticultural crops in the world. Nowadays, it become more and more popular among people for its higher nutrient value, abundant vitamins, minerals, and a variety of amino acids needed by the human body. However, high temperature (HT) promoted fruit respiration, which seriously affects the quality of grape after harvest (Min et al. 2001).

Abscisic acid (ABA) is a plant endogenous hormone with a sesquiterpene structure that exists in plants. With the deepening of research, ABA has been found to play an important role in plant drought, high salt, heat and other stress (Dar et al. 2017; Islam et al. 2018). As the sixth largest plant hormone, brassinolide (BR) plays an important role in regulating plant growth and development. In recent years, a lot of researches are carried out on the effect of BR on resisting plant biological and abiotic stress. Jin et al. (2015) found that spraying 24-EBR could increase antioxidant defense and glyoxalase systems under high-temperature stress. Kurepin et al. (2008) performed short-term high stress and BR treatments on canola seedlings, and the results showed that the enhancement of tolerance to HTS may be caused by endogenous ABA concentration. However, the effect of BR and ABA+BR on postharvest grape under HTS is rarely reported.

Metabolomics is an emerging omics technology following genomics, transcriptomics, and proteomics and has been widely used to connect genotype and phenotype (Fiehn 2002). Non-targeted metabolomic approach has been applied and developed for various research in grape. For instance, Negrel et al. (2018) use ultra-high-performance liquid chromatography (UHPLC) system coupled to high resolution mass

spectrometer (HRMS) based non-targeted metabolomic approach identify potential Plasmopara-specific metabolites in grape and revealed that lipids include ceramides, derivatives of arachidonic and eicosapentaenoic acid are not detected in healthy grapevine tissue. Jadhav et al. (2021) used non-targeted metabolomics approach revealed several biomarkers of gibberellic acid treatment metabolites, including arginine, proline, tyrosine, kaempferol, and so on in grape rachises, clusters, and berries after gibberellic acid treatment, and found that gibberellic acid treatment greatly affected polyphenols metabolites and amino acid metabolism.

In the current study, we investigated the response mechanism of ABA and BR treatments alone and together to HTS in fruit at the metabolome level. This study will provide a more theoretical basic and new research perspective according to the effects of ABA and BR on grapes.

Materials And Methods

2.1 Plant materials

Grapevine (*Vitis vinifera*) fruit of cultivar 'Shine Muscat' (Vitis labrusca× V. vinifera) grapevine 8-year old were collected from a commercial vineyard during the 2020 season. Approximately 40 berries from at least 10 bunches were tagged, and collected at ripening stage (14 weeks after flowering). We selected plant materials using phenotypic observation and quality determination. The samples were treated with 0.01 % Triton X-100 and 10 μM ABA, 10 μM BR or 10 μM ABA+10 μM BR (Sigma) solution, sprayed once every day for a total of 3 times. After the last treatment, they were put under 42 ℃ for high temperature stress, and then all samples were pictured every 12 h and frozen in liquid nitrogen and stored at -80 °C until analyzed. The 36-h sample was used for metabolite content determination.

2.2 Determination of soluble solids concentration, fruit weight, soluble sugars content, organic acids content and volatile compounds

Soluble solids concentration was determined using an Atago PR-100 refractometer (Atago Co. Ltd., Tokyo, Japan). Fruit weight was recorded using electronic balance. Soluble sugar and organic acid contents were determined using high-performance liquid chromatography (HPLC) (Zhang et al., 2018). Volatile compound production was measured using headspace solid-phase micro-extraction and gas chromatography–mass spectrometry as described by Jia et al. (2020).

2.3 Microscope observation

The fruit collected at 36 h with hormones treated and untreated were used for the transmission electron microscopy (TEM) observation. Each fruit was sliced with a razor blade into transverse sections $1mm^3$ into an EP tube with fresh TEM fixative. Then the following procedure referred to the method of Bonomelli et al. (2019).

2.4 Metabolites extraction

Grape fruit samples (0.1 g) were individually grounded with liquid nitrogen and the homogenate was resuspended with prechilled 80 % methanol and 0.1 % formic acid by well vortex. The samples were incubated on ice for 5 min and then were centrifuged at 15,000 g, 4 °C for 20 min. Some of supernatant was diluted to final concentration containing 53 % methanol by LC-MS grade water. The samples were subsequently transferred to a fresh Eppendorf tube and then were centrifuged at 15000 g, 4 °C for 20 min. Finally, the supernatant was injected into the liquid chromatography-mass spectrometry (LC-MS/MS) system analysis.

2.5 Ultra-High Performance Liquid Chromatography-Mass Spectrometry (UHPLC-MS/MS) analysis

UHPLC-MS/MS analyses were performed using a Vanquish UHPLC system (Thermo Fisher, Germany) coupled with an Orbitrap Q Exactive^{TM} HF mass spectrometer (Thermo Fisher, Germany) in Novogene Co., Ltd. (Beijing, China). Samples were injected onto a Hypesil Goldcolumn (100 × 2.1 mm, 1.9 μm) using a 17-min linear gradient at a flow rate of 0.0002 L·min^{-1}. The eluents for the positive polarity mode were eluent A (0.1 % FA in Water) and eluent B (Methanol). The eluents for the negative polarity mode were eluent A (5 mM ammonium acetate, pH 9.0) and eluent B (Methanol). The solvent gradient was set as follows: 2 % B, 1.5 min; 2-100 % B, 12.0 min; 100 % B, 14.0 min; 100-2 % B, 14.1 min; 2 % B, 17 min. Q Exactive^{TM} HF mass spectrometer was operated in pos/neg polarity mode with spray voltage of 3.2 kV, capillary temperature of 320 °C, sheath gas flow rate of 40 arb and aux gas flow rate of 10 arb.

2.6 Data processing and metabolite identification

The raw data files generated by UHPLC-MS/MS were processed using the Compound Discoverer 3.1 (CD3.1, Thermo Fisher) to perform peak alignment, peak picking, and quantitation for each metabolite. The main parameters were set as follows: retention time tolerance, 0.2 min; actual mass tolerance, 5 ppm; signal intensity tolerance, 30 %; signal/noise ratio, 3; and minimum intensity, 100,000. After that, peak intensities were normalized to the total spectral intensity. The normalized data was used to predict the molecular formula based on additive ions, molecular ion peaks and fragment ions. And then peaks were matched with the mzCloud (https://www.mzcloud.org/), mzVault and MassList database to obtain the accurate qualitative and relative quantitative results. Statistical analyses were performed using the statistical software R (R version R-3.4.3), Python (Python 2.7.6 version) and CentOS (CentOS release 6.6). When data were not normally distributed, normal transformations were attempted using of area normalization method.

2.7 Data analysis

These metabolites were annotated using the KEGG database (https://www.genome.jp/kegg/pathway.html), HMDB database (https://hmdb.ca/ metabolites) and LIPIDMaps database (http://www.lipidmaps.org/). Principal components analysis (PCA) and Partial least squares discriminant analysis (PLS-DA) were performed at metaX (a flexible and comprehensive software for processing metabolomics data). We applied univariate analysis (t-test) to calculate the statistical significance (*P*-value). The metabolites with VIP > 1 and *P*-value < 0.05 and fold change (FC) ≥ 1.5 or FC

≤ 0.667 were considered to be differential metabolites. Clustering heat map were normalized using z-scores of the intensity areas of differential metabolites and were plotted by Heatmap package in R language. The functions of these metabolites and metabolic pathways were studied using the KEGG database. The metabolic pathways enrichment of differential metabolites was performed, when ratio was satisfied by x/n > y/N, metabolic pathway was considered as enrichment, when *P*-value of metabolic pathway < 0.05, metabolic pathway was considered as statistically significant enrichment.

2.8 RNA extraction and quantitative real-time (qRT)-PCR

Total RNA was extracted from the grape using Quick RNA Isolation Kit (Hua Yue Yang, Beijing China). The total RNA was reversely transcribed into cDNA according to HifairTM 1st Strand cDNA Synthesis SuperMix Kit for qRT-PCR (gDNA digester plus) (Yeasen, Shanghai, China). Specific primers were designed using online software Primer 3 Plus (http://primer3.ut.ee/) (Table S1). Vv18S was used as internal reference gene. TaKaRa SYBR Premix Ex Taq™ II (Takara, Dalian, China) was used to perform qPCR on ABI prism 7900 Real-Time PCR system (Applied Biosystems, USA). Each reaction system was 200 nM gene-specific primer and 1.0 μl cDNA template, 5 μl TaKaRa SYBR Premix Ex TaqTM II, the final volume was 10 μl. The qRT-PCR cycle parameters were set to 95 °C for 2 min, followed by heating at 95 °C for 10 s and annealing at 60 °C for 40 s for a total of 40 cycles. 3 replicates for each sample.

Results

3.1 Physiological changes under high temperature

To observe the influence of different hormones on grape fruit quality under HTS, fruit color, total soluble solid concentrations (SSC) content and fruit weight were measured at different times (Fig.1A, Table 1). The color of all the groups has no significant difference at 12 h after heat treatment. BR-treated grape became brown first comparing to other groups, but at 36 h the browning was deeper in the control (CK) than other hormone treatment groups. ABA treated grape has a better appearance than other treated groups. SSC content of fruit increased gradually from 12 h to 36 h and then decreased after HT treatment, while BR treated fruit has a higher increasement at 24 h. The rate of increment in ABA, BR and ABA+BR treated fruit were higher than that of in CK. Meantime, ABA treated fruit has the highest increment rate from 12.80 to 14.37 %, and then was ABA+BR-treated fruit. The weight of fruit under different treatment has no significant change.

3.2 Soluble sugar and organic acid content

To determine the changes of fruit internal quality under different treatments, soluble sugar content and organic acid content were measured. As shown in Fig.1, hormone treatments promoted the accumulation of soluble sugar content, and ABA treated group was higher than BR and ABA+BR treated groups. Sucrose was only found under ABA treatment at 12 h. Glucose and fructose content under ABA treatment were significantly increased at 24 h and 36 h, while ABA+BR treated grape was at 36 h and BR treated grape was at 48 h. Tartaric acid is the main organic acid in grape and BR treated grape contained higher

organic acid content at 12 h than other groups (Fig.S1). The total organic acids in AB+BR treated group gradually increased from 12 h and the content was higher than CK group from 36 h. The change of organic acids was not significant in ABA treated grape, but markedly decreased after 12 h in BR-treated group and then increased to the same level as CK and ABA treated groups at 48 h.

3.3 Volatile compounds

In grape, a total of 117 volatiles compounds, including alcohols, benzenoids, aldehydes, acids, ketones, alkanes, olefins, and esters, were detected in the four treatment groups (Table S2; Fig. S2). There were 75 compounds identified in ABA-treated grape, 70 in ABA+BR-treated grape, 71 in BR-treated grape, and 69 in the CK. Twenty-eight alcohols were detected in 16 samples of four different times and accounted for 36.87 % – 73.26 % of the total volatile compounds. Among these compounds, the content of acetoin and linalool was higher. The content of alcohol components treated by hormones was lower than that of in CK, but higher at 48 h after ABA+BR treatment. Geraniol was only found in ABA and ABA+BR treated grape and contained a higher content. Eighteen aldehydes were detected in grape, accounting for 9.40 % – 48.73 % of the total volatile compounds. Among which. hexanal was the main aroma component and the highest content was in ABA-treated grape at 12 h, accounting for 32.50 %. The content of 2-hexenal at 12 h treatment with ABA and ABA+BR was both higher than CK. After treatment, 6 acids were detected in grape fruit and some of which were only found in once. In addition, 9 aromatic benzene compounds were found in grape and accounted for 0.31 % – 4.35 % of all the volatile compounds. Among them, benzaldehyde was only found in ABA treated and untreated. Although hormone treatment decreased the content of ketones and olefins, more types of them were found in ABA and BR treated grapes. Similarly, more types of alkanes, such as isobutane, butane, and undecane, were found in ABA-treated grape. Seven esters accounting for 0.61 % – 9.63 % were detected in grape, among which the higher content was ethyl acetate in ABA+BR treated grape.

3.4 Cell ultrastructure

To determine the effect of hormones treatment on grape fruit under HTS, ultrastructure analysis was performed by transmission electron microscopy (TEM). The cell structure had significant differences under different treatments (Fig. 2). The cell shape changed greatly in CK under HTS. Cell wall structure was loosened, showing obvious flocculation, and middle lamella was also found to be severely disintegrated. The cellulose wall has a small amount of disintegrated material diffused and the cell had severe plasmolysis. Comparing to CK, the microfibrils composed of cellulose were arranged more neatly, and the middle lamella was clear under ABA treatment, though microfibril had slightly degradation under BR treatment and middle lamella was partially disintegrated under ABA+BR treatment.

3.5 Metabolic profiles

To determine the response of grape fruit to HTS after different hormone treatments at the metabolic level, we identified the alteration of metabolites in grape fruit using LC-MS. After data processing, 549 and 201 metabolites were detected in LC-MS/MS pos mode and neg mode, respectively. All data were analyzed by

PCA to investigate whether sample groupings by treatments were formed (Fig. S3). The PCA plot showed a separation between BR and ABA+BR treated grape and CK grape.

Then we determined differences between hormone treated groups and CK group by supervised PLS-DA analysis. The results showed that ABA, BR and ABA+BR treatment and CK were separated well by PC1 under both pos and neg mode (Fig. 3). According to their VIP scores, PLS-DA identified 20 dominant components that were contributed to separation in the pos and neg mode (Fig. 4). Among them, the most significant ones in ABA vs. CK were phlorizin, bruceine D, phloretin, hexadecanamide, D-glucono-1,5-lactone, and geniposidic acid (Fig. 4A and D). In the BR vs. CK, the most remarkable ones were D-proline, irisflorentin, indole-3-acrylic acid, heroin-d3, thromoboxane B1, 2-(1H-1,2,3-benzotriazol-1-yl)-N-(2,3-dihydro-1H-inden-2-yl) acetamide, and yuheinoside (Fig. 4B and E). Gamma, gamma-dimethylallyl pyrophosphate, abscisic acid, N-(4-butyl-2-methylphenyl)-N'-[4-(4-methylpiperazino) phenyl] urea, kaempferol, trifolin, (±)-abscisic acid, and (5ξ,9ξ)-17-Hydroxykaur-15-en-19-oic acid were the remarkable metabolites in the ABA+BR vs. CK (Fig. 4C and F).

3.6 Identification of differential metabolites

To determine which compounds were involved in the metabolic process under HTS after different hormone treatment compared to the CK, univariate statistical analysis by t-test ($P < 0.05$), fold change (FC) > 1.5 or < 0.667 and variable importance in the projection (VIP) >1 was used to select differential metabolites.

Compared with CK, 47 differential metabolites (25 of which were not matched by HMDB) in pos mode and 20 (5 of which were not matched by HMDB) in neg mode were identified in ABA treated grape (Fig. S4; Table S3). Among the metabolites (by HMDB), most of them were involved in flavonoids (6 metabolites in total, 4 of which were up-regulated and 2 of which were down-regulated), followed by carboxylic acids and derivatives (5 metabolites in total, 4 up-regulated and 1 down-regulated) and other related metabolites were identified.

70 differential metabolites (51 of which were not matched by HMDB) in pos mode and 18 (11 of which were not matched by HMDB) in neg mode were identified in HTS after BR treatment compared to CK (Fig. S5; Table S4). Generally, carboxylic acids and derivatives (7 metabolites in total, 4 of which were up-regulated and 3 of which were down-regulated) contained the great number of metabolites (by HMDB), followed by fatty Acyls (5 metabolites in total, 4 of which were up-regulated and 1 of which was down-regulated), 2 phenols, and 2 prenol lipids were identified.

After ABA+BR treatment, 140 differential metabolites (66 of which were matched by HMDB) in pos mode and 70 differential metabolites (27 of which were matched by HMDB) in neg mode were identified compared to CK (Fig. S6; Table S5). In general, the largest number of metabolites (by HMDB) among these differential metabolites was carboxylic acids and derivatives (20 metabolites in total, 10 of which were up-regulated and 10 of which were down-regulated), followed by fatty acyls (16 metabolites in total,

2 of which were up-regulated and 14 of which were down-regulated), 9 prenol lipids, 6 flavonoids, and 4 benzene and substituted derivatives were identified.

3.7 Analysis of metabolic pathways

To determine which metabolic pathway differential metabolites were involved in, the metabolites were annotated to KEGG database. The results showed that 16 metabolic pathways in pos mode and 22 in neg mode were enriched in ABA vs. CK (Table S6). Among the 38 metabolic pathways, most of them were mainly involved in amino acid metabolism, such as tryptophan metabolism, phenylalanine, arginine and proline metabolism, arginine biosynthesis, followed by sugar metabolism (such as pentose phosphate pathway, glycolysis/gluconeogenesis, fructose and mannose metabolism). Top 20 metabolic pathways in pos and neg mode were displayed by bubble scatterplot (Fig. S7A and D). Among these metabolic pathways, sulfur relay system, and flavone and flavonol biosynthesis were significantly enriched with *P*-value ≤ 0.05 in pos mode, and in neg mode were biosynthesis of secondary metabolites and propanoate metabolism.

In BR vs. CK, 26 metabolites pathways were enriched in pos mode and 3 pathways were enriched in neg mode (Table S7). Among the 29 metabolic pathways, organic acid metabolism was the main one, such as alpha-linolenic acid metabolism, fatty acid metabolism, biosynthesis of unsaturated fatty acids, followed by amino acid metabolism (such as arginine and proline metabolism, cysteine and methionine metabolism, lysine degradation, tryptophan metabolism). Top 20 metabolic pathways enriched in pos and neg mode were showed by scatterplot (Fig. S7B and E). Arginine and proline metabolism, lipoic acid metabolism, alpha-linolenic acid metabolism, and plant hormone signal transduction were remarkable enriched pathways.

Thirty-seven metabolites pathways were found enriched in pos mode and thirty pathways enriched in neg mode after ABA+BR treatment compared with CK (Table S8). Among the 67 metabolic pathways, organic acid metabolism was the main one, such as arachidonic acid metabolism, alpha-linolenic acid metabolism, glyoxylate and dicarboxylate metabolism, fatty acid biosynthesis, linoleic acid metabolism, butanoate metabolism, fatty acid metabolism, followed by amino acid metabolism and sugar metabolism. Metabolic pathways of top 20 enriched in pos and neg mode were showed by bubble scatterplot (Fig. S7C and F). The most enriched pathways in pos mode were purine metabolism, glutathione metabolism, zeatin biosynthesis and arachidonic acid metabolism, while in neg mode were sugar metabolism (galactose metabolism, glycolysis/gluconeogenesis, starch and sucrose metabolism), and biosynthesis of amino acids.

3.8 Stress associated genes expression under high temperature

To determine the effect of hormones on grape fruit under HT, the expression of antioxidant and HSPs related genes were selected for qRT-PCR (Fig. 5). Results showed that the expression level of *VvSOD* was higher in hormone treated groups than in CK, and ABA+BR treated group maintained a high level at different times, while *VvPOD* was lower than CK. *VvCAT* expression level decreased with time and was

lower in CK than others after 12 h. The expression level of *VvHsf A2, VvGols1* and *VvHSP 17.9* decreased with time and were higher in ABA, BR and ABA+BR treated groups than that of in CK.

Discussion

Temperature stress, especially HTS, is one of the abiotic stress factors that affect the yield and quality of grapes. The entire growth and development of the grapes will be affected by HT, especially during ripening (Luo et al. 2011). In this study, we explored the effect of ABA, BR and ABA+BR treatments on grape fruit quality under HT through metabolomics. The results showed that hormone treatment alleviate HTS and single hormone treatment was better than two hormone combination treatments.

4.1 Hormone treatment alleviates the decline in fruit quality

ABA, BR and ABA+BR treatment result in better visual quality under HT than that of in CK (Fig. 1A). The result suggested that ABA and BR played important roles in plant under HTS (Kurepin et al. 2008; Islam et al. 2018). Our study, however, was focused on the effects of single hormones and a combination of hormones on grape fruit under HTS. There was no significant difference between treated grape and untreated grape at 12 h, while at 36 h, hormone treated groups contained better appearance and ABA treatment contained the best.

Hormone treatments had positive influences on SSC accumulation of grape fruit (Table 1). Increased SSC at HT is probably due to degradation of macromolecular substances. The SSC content of grape fruit in all treatments under HT reached its peak at 36 h, except BR treated grape which increased continuously to 48 h. The increase of SSC was larger after hormones treatment than CK, and ABA treated grape was the largest. Our results were consistent with previous study that exogenous ABA or BR can cause a higher content of SSC (Wang et al. 2016). The weight of the fruit was decreased with time during the HT treatment but had no significant difference between hormones treatment and CK (Table 1) which might be related to grape variety and processing time.

Sugar and acid contents are an important indicator of grape fruit quality. Research had confirmed that exogenous 24-EBR can effectively promote the accumulation of soluble sugar in grape (Xu et al. 2015). In our study, exogenous ABA, BR and ABA+BR treatment promoted the accumulation of soluble sugar content and degradation of fruit sucrose. The time of significant increase was at 24 h in ABA treatment, 36 h in ABA+BR treatment and 48 h in BR treatment, respectively (Fig. 1B-D). These results suggested that exogenous ABA, BR and ABA+BR treatment could enhance the accumulation of soluble sugar content under HT and the promotion effect of ABA treatment was earlier than others.

Organic acids of grape fruit gradually decreased after BR treatment and then increased at 48 h, which was contrary to the CK (Fig. S1). The decreased organic acids might be due to a degradation of the acids into raw materials for the synthesis of other substances (Ruffner et al. 1976). ABA treatment maintained a steady level for the 36 h, which suggested that exogenous ABA delayed the degradation of organic

acids. In addition, ABA+BR treatment promoted the accumulation of acids. These results might be caused by hormone treatment slowing down the respiration rate of grape under HT.

Temperature and storage time are important factors affecting the flavor quality of postharvest fruit (Kader 2008). Under different hormone treatments, ABA treated fruit contained more volatile components than others (Table S2). Alcohols and aldehydes are the main components of the aroma of 'Shine Muscat' and the content of aroma changed under different storage times. 'Shine Muscat' is a major table grape cultivar in Japan, which has a strong muscat flavor (Yamada et al. 2008). Researches have reported that linalool, geraniol, α-terpineol, and citronellol are major terpenols that contribute to the muscat flavor of muscat grape cultivars (Ribéreau-Gayon et al. 1975). In our study, the production of linalool, alpha-terpineol and citronellol were promoted by HT and significantly promoted by ABA+BR for the first 24 h. Meanwhile, linalool content was decreased with storage time under ABA and BR treatment (Fig. S2; Table S2). In addition, hexanal and 2-hexenal, which contained the fragrance of grass, were significantly promoted by hormones during short-term storage.

The integrity of the cell wall structure plays an important role in plant immunity and maintaining fruit quality. The devaluation of fruit firmness after harvest is mainly due to the disruption of the middle lamella and the change of primary cell wall composition (Brummell 2006). In our study, cell wall structure was severely damaged, showing loose structure and disappearance of middle lamella under HT (Fig. 2), suggesting that HT severely destroyed the cell wall structure of grape. However, microfibril and middle lamella of fruit were clearly visible and arranged neatly under ABA treatment. Although the microfibril of the cell wall partially disappeared under the treatment of BR and ABA+BR, the middle lamella remained relatively intact, which indicated that exogenous ABA, BR and ABA+BR treatment could reduce cell wall damage under HT.

Antioxidative systems play a crucial role in protecting plants from the damage ROS under environmental stresses. SOD, POD and CAT are important antioxidant enzymes, which play coordinated functions in processing ROS (Halliwell 1974). In our study, the expression level of *VvSOD* and *VvCAT* were decreased with time under HTS, which was consistent with previous studies that CAT level and SOD activity were dropped under heat stress (Foyer et al. 1997; Liu and Huang 2000). Among them, *VvSOD* level was higher than that of in CK after hormone treatments, which suggested that SOD played positive effect in processing ROS under HTS after ABA, BR and ABA+BR treatment. Heat stress transcription factor (Hsf) A2 and B1, galactinol synthase (Gols)1 and HSP17.9 play crucial roles in heat shock stress (Panikulangara et al. 2004; Khan and Shahwar 2020). In our study, the expression level of *VvHsf A2, VvGols 1* and *VvHSP 17.9* were all higher in hormone treated groups than that of in CK, especially *VvHSP 17.9,* while *VvHsf B1* was higher in CK after 24 h. Among the three treated groups, *VvHsf A2* and *VvHSP 17.9* were higher in ABA and BR treated groups and *VvHsf B1* was higher in ABA+BR treated group (Fig. 5). These results indicated that all the three groups promoted the expression of heat shock related genes.

4.2 Metabolic mechanism under hormone treatments

Plant resistance is a complex physiological and metabolic process, which involves different kinds of metabolites such as soluble sugars, amino acids, organic acids, antioxidants and lipids (Rontein et al. 2002; Kaplan et al. 2004; Xu et al. 2006). In our study, the metabolic pathway analysis (Table S3 and Table S6) showed that many pathways related to organic acid metabolism, amino acid metabolism, sugar metabolism and secondary metabolites were significantly changed after hormone treatments.

Amino acids not only play a key role in the osmotic regulation of plants in response to abiotic stress, but also serve as precursors of a large number of metabolites, with multiple functions to deal with various stresses (Rontein et al. 2002). Specifically, compared with CK, the content of glutamic acid increased over 1.72-fold after ABA treatment and 1.86-fold after ABA+BR treatment under HTS. Glutamate is the central position in amino metabolism and used to produce other amino acids, such as proline which is considered to be an important metabolite in response to abiotic stress (Kishor and Sreenivasulu 2014). In addition, other amino acids, such as S-adenosyl-L-methionine (2.34-fold) and tryptophan (1.71-fold) after ABA treatment, Phe-Phe (2.74-fold) and gamma-Glu-Leu (2.75-fold) after BR treatment, and aspartic acid (2.15 fold), L-serine (1.86-fold) and glutamine (1.86-fold) after ABA+BR treatment were significantly accumulated in grape fruit, which played a role in regulating osmotic pressure in response to HTS (Kaplan et al. 2004).

Studies have suggested that soluble sugars, as important compatible components, played crucial roles in promoting plants heat tolerance (Wahid and Close 2007). Kaplan et al. (2004) found that HT promoted the accumulation of carbohydrate, such as maltose, sucrase, raffinose etc. in Arabidopsis. In our study, compared with CK, D-raffinose (1.63-fold) in ABA treated grape, uridine 5'-diphosphogalactose (27.27-fold), N-acetylglucosamine 1-phosphate (17.18-fold), α,α-trehalose (6.21-fold), sucrose (5.06-fold), α-D-Mannose 1-phosphate (3.89-fold) and D-raffinose (2.38-flod) in ABA+BR treated grape were all significantly increased under HTS. While the downregulated metabolites include sugar alcohol (D-(-)-mannitol, 0.18-fold; dulcitol, 0.39-fold; maltitol, 0.18-fold) and sugar acid (gluconic acid, 0.66-fold) in ABA+BR treated grape. These results indicated that more accumulation of soluble sugars was induced by exogenous ABA and ABA+BR under HTS.

Secondary metabolites are considered to be physiologically important particularly in stress tolerance. Phenolics, including flavonoids, anthocyanins, phenylpropanoids, etc., are class of important secondary metabolites and played crucial role in resisting abiotic stress (Dixon and Paiva 1995; Rivero et al. 2001). In this study, most flavonoids, including epicatechin (6.08-fold), phloridzin (4.57-fold), phlorizin (3.71-fold), astilbin (1.87-fold) in ABA treated grape, and apigenin (1.77-fold) in BR treated grape, classified by HMDB were significantly up-regulated, while only two flavonoids (naringenin, 7.25-fold; epicatechin, 4.64-fold) in ABA+BR treated grape were up-regulated. In addition, some terpenoids, such as pterosin G (1.71-fold), pteroside D (1.94-fold) and warfarin sodium (1.53-fold) in ABA treated grape, and integrifoside A (3.13-fold) in BR treated grape were also increased, which could improve plant tolerance to temperature stress (Copolovici et al. 2012).

Hormones played an important role in plant monitoring and adaptation to adverse environmental conditions and hormone content and biosynthesis are changed under heat stress (Maestri et al. 2002; Wahid et al. 2007). Our study showed that EBR (6.10-fold), abscisic acid (2.60-fold) in pos mode and (±)-abscisic acid (2.60-fold) in neg mode remarkably increased after BR treatment. Additionally, EBR (8.27-fold), abscisic acid (7.61-fold) in pos mode and (±)-abscisic acid (6.24-fold) in neg mode were also significantly induced by ABA+BR. EBR plays a pivotal role in various physiological processes as a plant growth regulator, and also enhances plant tolerance to a variety of abiotic and biotic stresses (Xi et al. 2013; Jia et al. 2015; Sharma et al. 2016). Improving the antioxidant capacity of plants is an important way for exogenous EBR to improve plant resistance (Wani et al. 2019). In addition, ABA, as stress hormones, has been reported to play an important role in the heat tolerance of plants (Daie and Campbell 1981; Abass and Rajashekar 1993; Liu et al. 2019). Kurepin et al. (2008) confirmed that exogenous BR can induce endogenous ABA concentration under HTS conditions, which was consistent with our study.

Conclusion

The finding of the present study suggested that ABA, BR and ABA+BR have a good impact on maintaining grape fruit quality under HTS. ABA, BR and ABA+BR treatment delayed the browning of fruit color and promoted the accumulation of SSC and soluble sugar content. ABA and ABA+BR could reduce the damage of HTS to plants by accumulating more amino acid, carbohydrate and secondary metabolites. BR responded to HTS by promoting the accumulation of amino acids and secondary metabolites. BR and ABA+BR treatment also increased the content of endogenous ABA and EBR. In addition, ABA, BR and ABA+BR treatment delayed the degradation of the middle layer and microfibers and promoted the expression level of antioxidant and heat shock related genes. From above findings, it could be concluded that ABA, BR, ABA+BR could be used to delay high-temperature damage and maintain grape fruit quality.

Declarations

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Author contributions

LJH and DTY treated the samples and conducted data analysis and drafted the manuscript. ZT and ZYP helped data analysis. FJG helped to revise the manuscript. YK helped to draft the manuscript. DTY

participated in the design of the study. JHF designed and coordinated this study and revised the manuscript. All authors read and approved the final manuscript.

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Statements and Declarations

The authors declare no conflict of interest.

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Tables

Table 1 Soluble sugar content and fruit weight were affected by high temperature. Soluble sugar content was enhanced by ABA and BR; high temperature has not effect on grape weight within 48 h. CK, control; ABA, abscisic acid, BR, brassinolide (*n* = 3; ±SD).

		CK	ABA	BR	ABA+BR
Soluble solids concentration (%)	12 h	13.33±0.68	12.80±0.12	13.47±0.47	13.03±0.22
	24 h	13.63±0.82	13.40±0.41	13.97±0.77	13.30±0.12
	36 h	14.23±0.42	14.37±0.17	13.83±0.34	14.23±0.43
	48 h	14.10±0.56	14.27±0.08	14.30±0.29	14.03±0.39
Weight (g)	12 h	65.77±0.2	62.74±0.3	69.39±0.23	65.87±0.15
	24 h	65.55±0.39	62.53±0.4	69.18±0.32	65.65±0.32
	36 h	65.3±0.12	62.33±0.48	68.99±0.18	65.46±0.21
	48 h	65.23±0.23	62.26±0.22	68.89±0.36	65.39±0.22

Figures

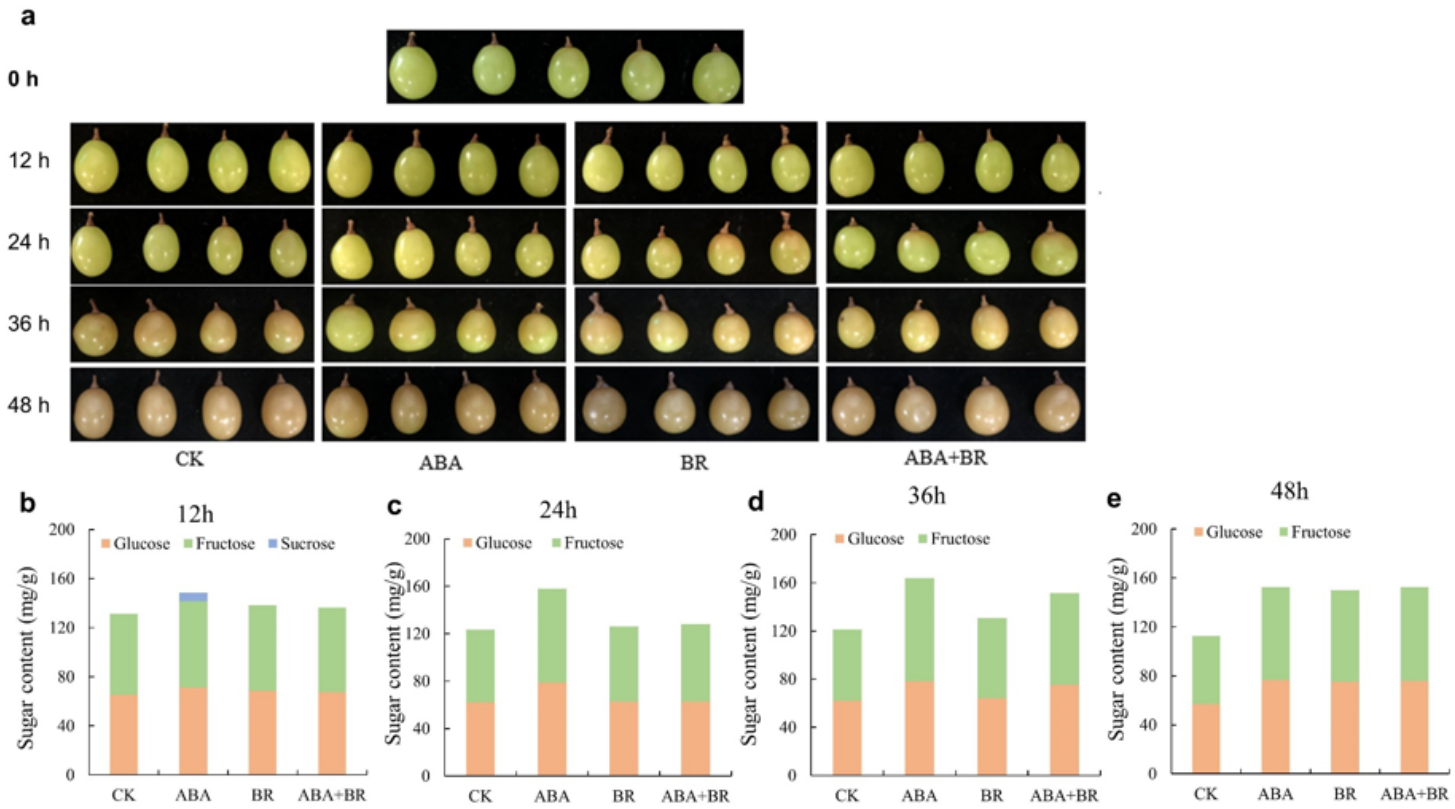

Figure 1

Hormone treatment delayed the browning of fruit and enhanced soluble sugars of the fruit. a Hormone treatment delayed fruit browning and ABA treated grape was much better. b-e Soluble content was promoted by hormones and ABA-treated grape increased first. CK, control; ABA, abscisic acid, BR, brassinolide.

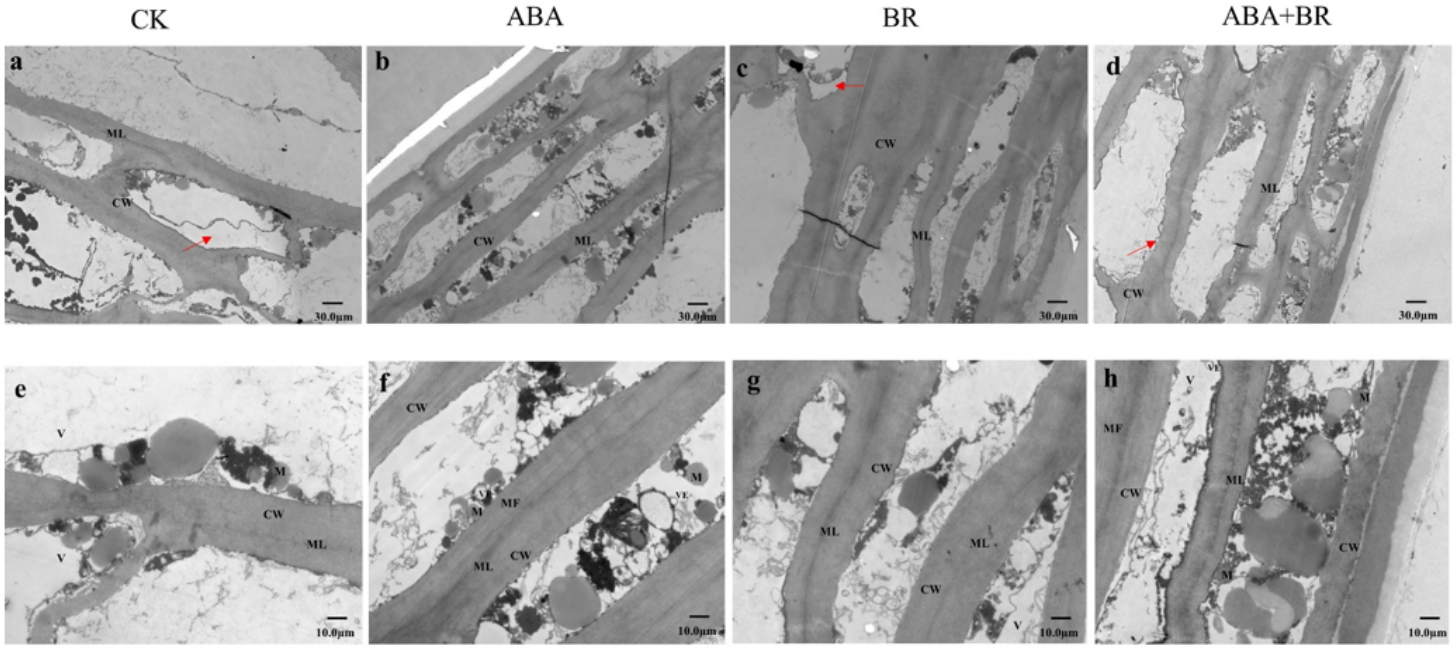

Figure 2

Hormone treatment delayed the destruction of cell structure. a, e Cells showed obvious plasmolysis (shown by red arrow) and middle lamella was also found to be severely disintegrated. b, f Cell structure was intact, and middle lamella and microfibrils composed of cellulose were clear. c, g Middle lamella was partial degraded and microfibrils almost disappeared. d, h Cell wall begun to appear plasmolysis (shown by red arrow). Part of the middle lamella of the cell wall was degraded but the middle layer of the cell was clear. CW, cell wall; M, mitochondrion; V, vacuole; ML, middle lamella; VE, vesicles MF, microfibril; CK, control; ABA, abscisic acid, BR, brassinolide.

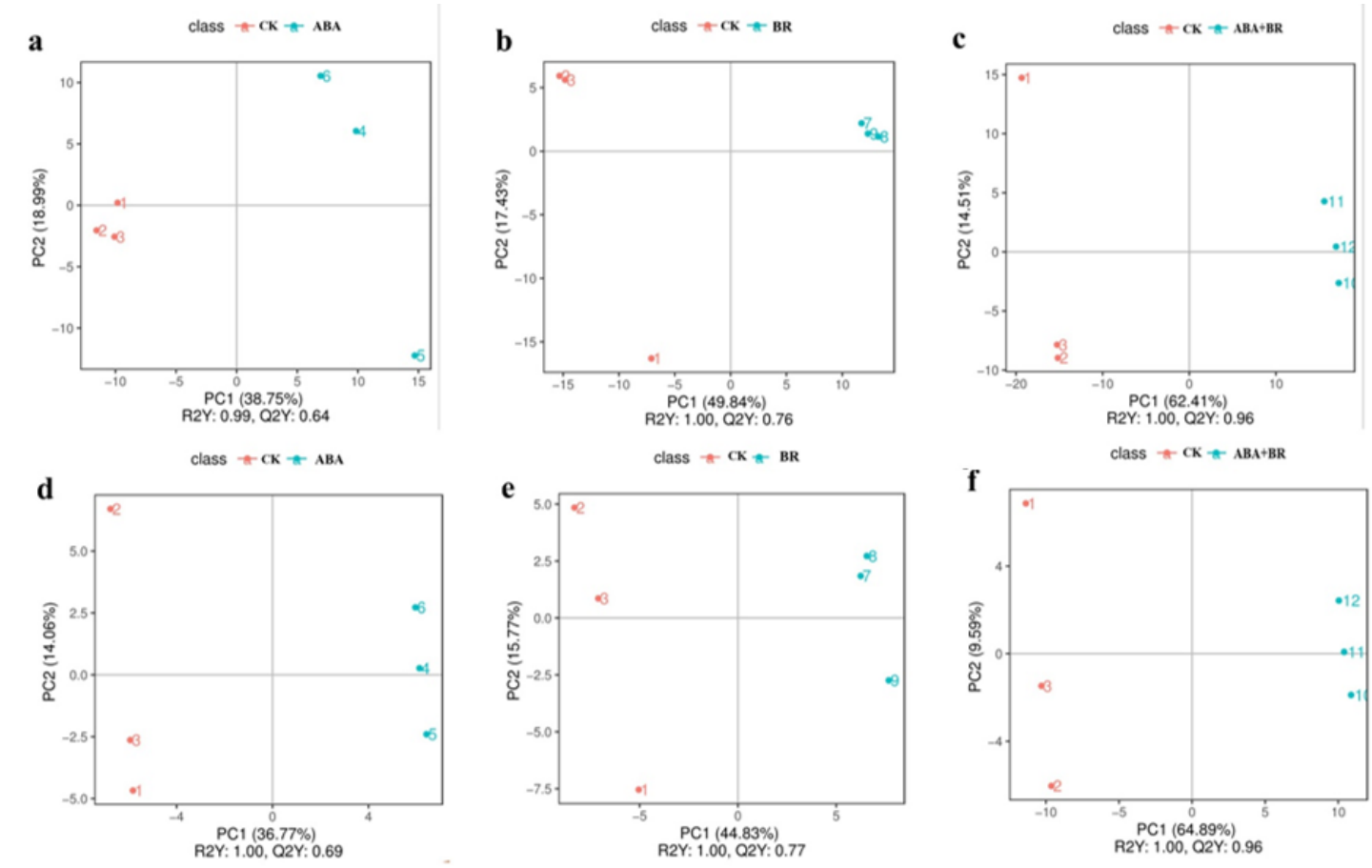

Figure 3

Scores plot of PLS-DA of metabolites extracted from fruit of grape after ABA, BR, ABA+BR and control treatment under high storage temperature. a, d ABA treated grape and control grape were clearly separated under pos and neg mode. b, e BR treated grape and control grape were clearly separated. c, f ABA+BR treated grape and control grape were clearly separated. CK, control; ABA, abscisic acid, BR, brassinolide.

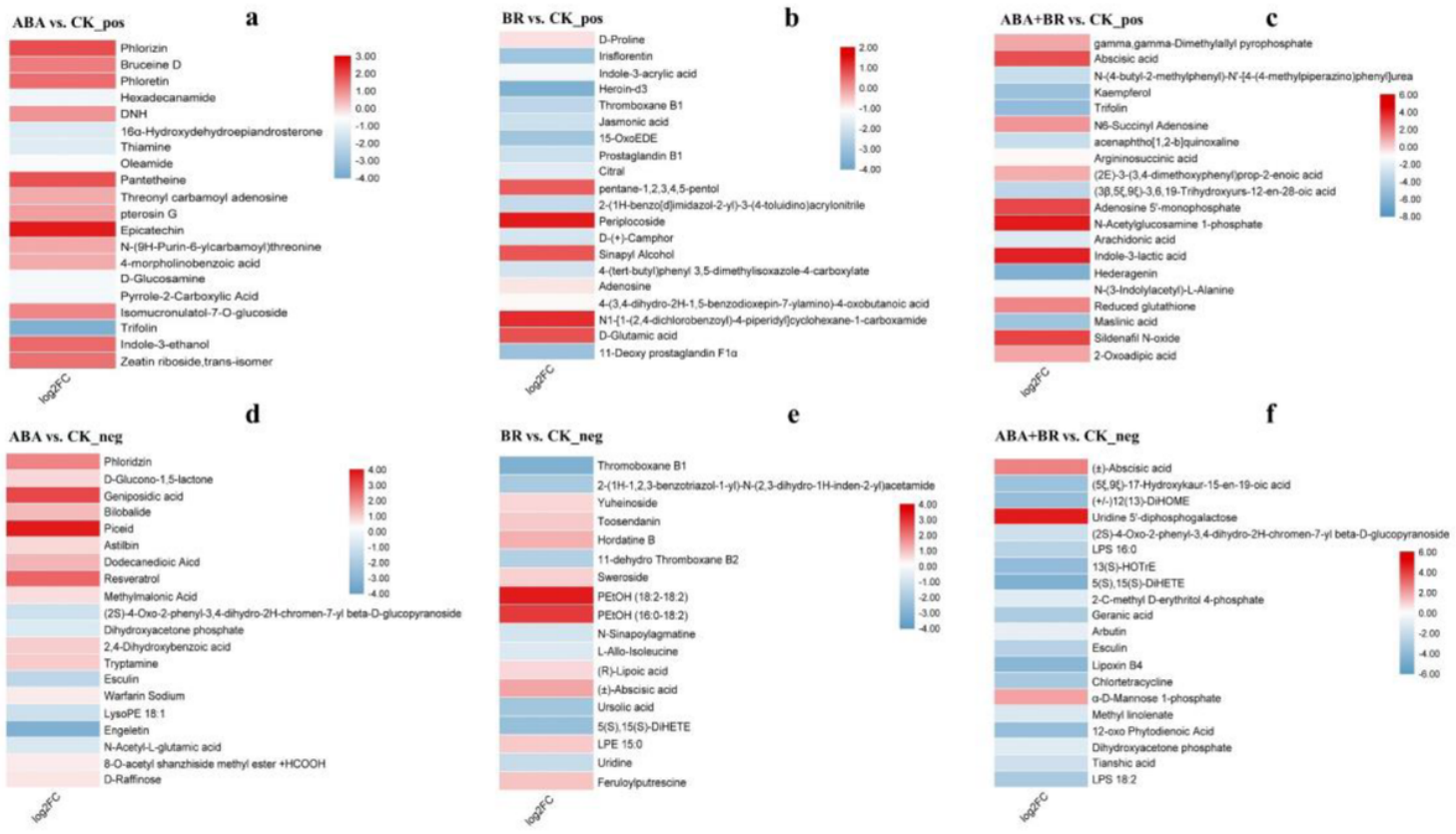

Figure 4

The fold change of top 20 differential metabolites of variable importance in the projection (VIP) scores in grape fruit of hormone treated compared to the control. a, d Heat map of differential metabolites after ABA treatment under pos and neg mode. b, e Heat map of differential metabolites after BR treatment under pos and neg mode. c, f Heat map of differential metabolites after ABA+BR treatment under pos and neg mode. CK, control; ABA, abscisic acid, BR, brassinolide.

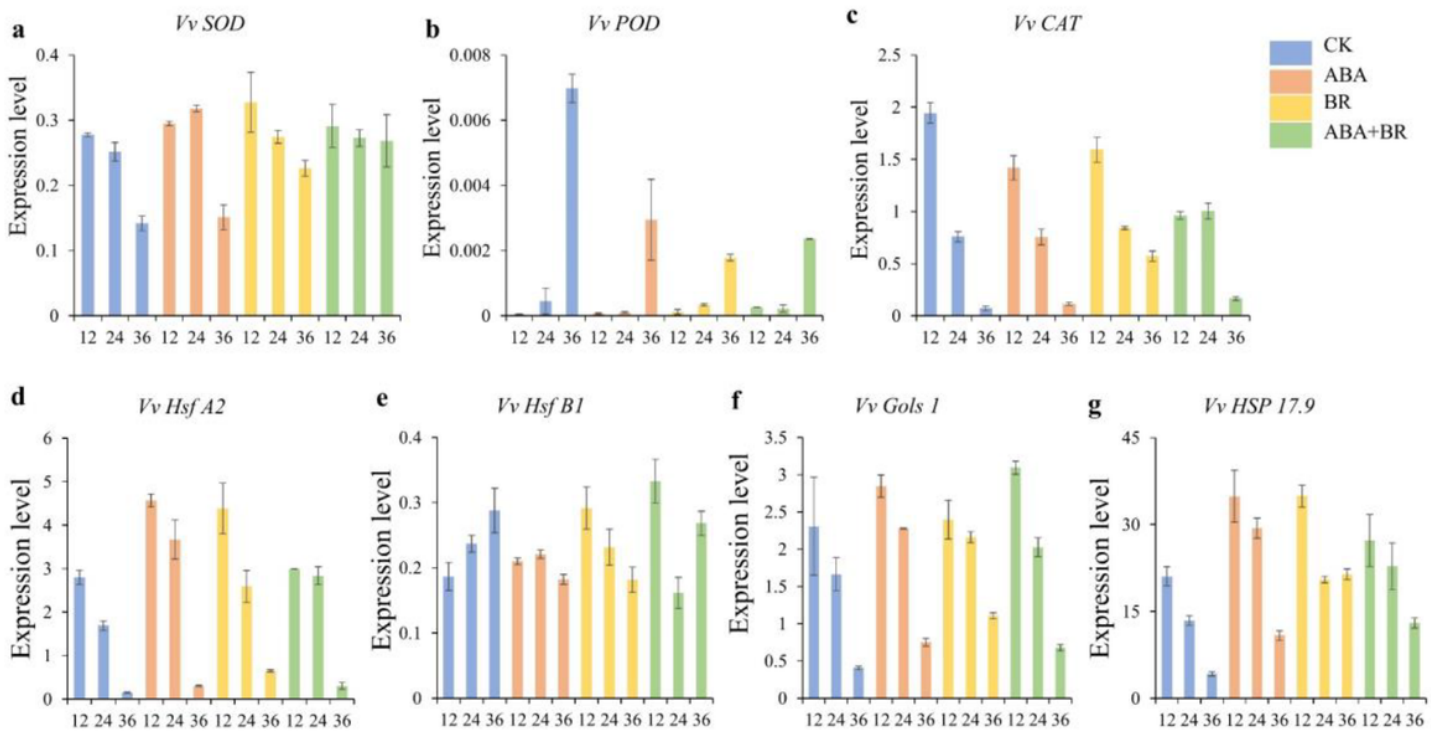

Figure 5

ABA, BR and ABA+BR treatment promoted the expression of antioxidative and heat shock related genes. a-c Expression levels of SOD, POD and CAT over time. d-g Expression levels of Hsf A2, Hsf B1,Gols1 and HSP17.9 over time. Data are expressed as means ± SD of three biological replicates. CK, control; ABA, abscisic acid, BR, brassinolide.

Supplementary Files

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- TableS7.xlsx
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