Effect of Exogenous NaCl On Anthocyanin Accumulation and Biosynthesis of Cabernet Sauvignon Grape Berries

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Abstract

Backgroud:

The coloring mechanism of grape (Vitis vinifera L.) berry in response to salinity during maturation is poorly understood. To determine the effect of salinity on fruit quality, especially anthocyanin accumulation, the grapevine cultivar Cabernet Sauvignon were sprayed with different concentrations of sodium chloride. Dynamic changes in anthocyanin accumulation and eight biosynthetic enzyme activities during maturation were also investigated to clarify the anthocyanin biosynthesis regulation.

Results

The analysis showed that the fruit's fresh weight was decreased by NaCl spray. But the grape quality including reducing sugar, soluble sugars, total phenols and tannins increased significantly on harvest time. Ten individual anthocyanins were detected from the skin of Cabernet Sauvignon by ultra performance liquid chromatography (UPLC). And there was a significantly greater accumulation of total anthocyanin contents under salt treatment. The NaCl spray provoked metabolic responses in grapes and enhanced biosynthetic enzyme activities during riping. Correlation analysis showed that anthocyanin accumulation was closely related to the key enzyme LDOX both in the control and 100 mM NaCl treatment berries.

Conclusion

The application of NaCl to grape foliage effectively increase the quality of the Cabernet Sauvignon grape, improving soluble sugar, organic acid, phenolics and tannin contents, and the total anthocyanin contents in grape skins after varasion. These findings provide novel insight into the crucial factors that directly modulate anthocyanin biosynthesis and consequently control grape coloration.

Backgroud

Grapevine (Vitis vinifera L.) is one of the most widely cultivated and economically important fruits in the world. Approximately 71% of annual yield is used for wine. The commodity value and market competitiveness of wine are affected by grape skin coloring degree and quality (Liang et al., 2008). The skin color is modified by anthocyanins synthesis and chlorophyll degradation (Sun et al., 2019). Anthocyanins are produced during secondary metabolism in grapevines and provide color and antioxidant activity to grape berries and wines (He et al., 2008). They also supply numerous health-promoting properties due to their potential benefit in anticarcinogenic properties and scavenging of reactive oxygen species (Hui et al., 2010).

Anthocyanin are primarily synthesized by phenylpropanoid and flavonoid pathways in plants, which requires the participation of two types of genes, structural and regulatory genes. The enzymes which directly participated in the synthesis and accumulation of anthocyanins are encode by the structural genes, which include chalcone synthase (CHS), phenylalanine ammonia-lyase (PAL), flavanone 3-hydroxylase (F3H), flavonoid 3'-hydroxylase (F3'H), flavonoid 3',5'-hydroxylase (F3'5'H), flavonol-4-reductase (DFR), flavonoids 3-O-glucosyltransferase (UFGT)and leucoanthocyanidin dioxygenase (LDOX). F3H hydroxylates flavanones to form dihydroflavonols. F3'H and F3'5'H are engaged in the biosynthetic pathway of cyanidin-based and delphinidin-based anthocyanins (Mato et al., 1998). DFR catalyses the conversion of dihydroflavonols to leucoanthocyanidins, followed by the the production of anthocyanidin from leucoanthocyanidins, which involves LDOX (Mori et al., 2005). And UFGT determines the color of grape berries (Davies et al., 1997). Regulatory genes include transcription factors *MYB, bHLH* (b helix-loop-helix), and *WDR* (trypthophan-aspartic acid repeat). They regulate the spatial and temporal expression of genes in the anthocyanin biosynthetic pathway by binding to the structural gene promoters (Ageorges et al., 2005; Holton and Cornish, 1995; Lecourieux et al., 2017). Anthocyanin biosynthesis are also affected by environmental factors such as fruit ripening (Singh Brar et al., 2008), sugar accumulation levels (González-SanJosé and Diez, 1992), abiotic stresses (Kyraleou et al., 2016; Lecourieux et al., 2017) and plant growth regulators.

Salinity stress is one of the most significant environmental stresses limiting agricultural production worldwide. Salinity is also an important issue for grapevine vegetative growth, because of its moderate sensitivity to saline environment. Studies have shown it stunts grape branches and decreases the yield components of grapevine (Walker et al., 2002). However, the application of salt stress during fruit production have been proposed to improve fruit quality. Mature tomatoes grown under salt stress have higher fructose and glucose concentrations, acid contents, and elevated amino acids (Dorais et al., 2001; Lu et al., 2010). Different salt concentrations could enhance the nutritional value of radish sprouts (Yuan et al., 2010), as well as sugar and acid content in citrus juice (B et al., 2007). A 40 or 80 mM NaCl solution decreased the quality parameters like sugar, soluble solids and organic acids of salt sensitive strawberry cultivars, Elsanta and Korona. In contrast, applying a 40 or 80 mM NaCl to the strawberries for 4 months increased the antioxidant capacity (i.e., superoxide dismutase, glutathione, phenols, and anthocyanins) and essential amino acids, particularly in Elsanta (Anna Keutgen, 2007). Tomatoes under moderate salt stress showed higher antioxidative capacity, relating to carotinoids and lycopene (D'Amico et al., 2003). Long-term moderate salinity treatment on Kyoho grape berry increased the soluble sugars, organic acids and anthocyanin content of grape fruits (Li et al., 2013).

However, the relationship between anthocyanin biosythesis and NaCl utilization has not yet been studied in wine grape. Therefore, the focus of the present study was to explore the influence of different salinity stress on berry quality and anthocyanin biosythesis under long-term application of NaCl solution to the Cabernet Sauvignon foliage.

Methods

Foliage treatments and berry sampling

The experiment was carried out in a *Vitis vinifera* L. cv Cabernet Sauvignon vineyard of the Great Wall Wine Co., Ltd in Penglai, Shandong Province, China (37°25'～37°50' N, 120°35'～121°09'E) during the 2015 vintage. The vines were planted in 2005, trained on a vertical trellis system and pruned using unilateral Royat cordon system. Six representative and consecutive rows were selected for the analysis. The grape leaves were sprayed with three different concentrations of NaCl solution (40, 60, 100 mM) every two weeks starting at 7 days after anthesis (DAA) until berry maturation respectively. At 90, 105 and 120 days after anthesis, 300 berries were taken randomly from three different positions in the sample clusters: the shoulder, middle and bottom. The berry size and fresh weight of the collected grapes were analyzed within 1 h after harvest . Berries collected for enzyme and biochemical analyses were immediately frozen in liquid nitrogen and kept at −80 °C until analysis.

Measurement of grape berry indexes

The total soluble solids (TSS) of collected berries was measured using a digital refractometer (TD-45, TOP Instrument, Zhejiang, China). And the titratable acidity was titrated with 0.1 M NaOH to an end-point at pH 8.3. Reducing sugar of the supernatant was analyzed by the dinitrosalicylic acid (DNS) method (L., 1959). Quantification of total phenolics was performed by the Folin–Ciocalteu's method (Minussi et al., 2003), using gallic acid as standard. While tannin quantification used Folin-Denis reagent, using catechinic acid as standard.

Soluble sugar and organic acid determination

The pulp was ground into powder with liquid nitrogen. And 3 g of powder was extracted three times in 6 ml of 80% (v/v) methanol (phosphoric acid for organic acid) and ultrasonic extraction for 20 min. The extract was concentrated with a rotary evaporator and filted with a 0.45 mm membrane filter. Then the filtrate was analysed using a HPLC System (SHIMADZU LC-20AT, Kyoto, Japan) to determine the soluble sugar and organic acid content, respectively.

Determination of anthocyanin profile

The extraction of anthocyanins was performed according to the method previously reported (Mattivi et al., 2006). The anthocyanin extraction was analyzed by UPLC-ESI-MS system (Waters Maldi Synapt Q-TOF, Shanghai, China). An aliquot of a 1 mL solution was injected into the UPLC column (2.1 × 100 mm ACQUITY UPLC BEH C18 column containing 1.7 μm particles) with a flow rate of 0.3 mL/min and operated at 45 °C. Buffer A consisted of 0.1% formic acid in acetonitrile, and buffer B consisted of 2% formic acid in water. Elusion procedures are: 0-20 min, 98-80 % B; 20-25 min, 80-20 % B; 25-26 min, 20-98 % B; 26-30 min, 98 % B. Mass spectrometry was performed using an electrospray source in positive ion mode, with a selected mass range of 20–1200 m/z. The ionization parameters were the following: 3.2 kV capillary voltage, source temperature at 100 °C, and desolvation gas temperature at 400°C. Malvidin-3-O-glucoside (Sigma-Aldrich, St. Louis, USA) was used as a common external standard, and other anthocyanin components were quantified by it.

Enzyme assay

The activities of key enzymes involved in anthocyanin biosynthesis were assayed by ELISA kit (Bangyi, Shanghai, China).

Statistical Analysis

All data were expressed as the mean ± SD of three replicates. Statistical difference between control and treated groups was analyzed by the LSD't- test at $P < 0.05$ using SPSS software (version 19.0, SPSS Inc., Chicago, IL, USA).

Results

General quality parameters of mature grape

The average fresh weight, transverse and vertical diameter of the berries decreased gradually with increasing salt concentrations. Berries sprayed with 40 mM NaCl had the lowest vertical diameter and fresh weight (Table 1). Both the reducing sugar and TSS content elevated under moderate salinity but declined under high salinity. The grapes under 40 mM NaCl treatment produced the highest levels of reducing sugar and TSS than in the control and other salt-treated berries (Table 1). However, the titratable acidity had the opposite trend (Table 1). Moreover, the salt treatment enhanced the total phenols and tannins of the berries significantly (Table 1).

Soluble sugars and organic acids

The grape berries mostly accumulate soluble sugars (fructose and glucose) during riping (Hilbert *et al.*, 2011). In the study, the salt-treated berries showed higher fructose and glucose concentrations than the control. And the most effective treatment was 40 mM NaCl (Table 2).

The concentrations of the four organic acids were also tested (Table 2). Tartaric and malic acid accounted for 46.6% and 48.1% in the control. While citric and oxalic acid just accounted for 2.84% and 2.52%. A significant increase of tartaric acid and oxalic acid was found under high salinity (100 mM NaCl) . And malic acid did not have a significant variation after NaCl spray. The percentage of tartaric acid increased whereas malic acid decreased under salt treatment., However, citric acid did not significantly changed in both content and percentage. In short, the sum of the four organic acids increased under 100 mM NaCl, which was mainly due to increased tartaric acid.

Effect of NaCl treatment on grape anthocyanins

In the test, a total of 10 individual anthocyanins were detected through UPLC analysis in ripe grapes. They are 5 non-acylated and 5 acylated individual anthocyanins, including Dp: Delphinidin-3-glucoside, Cy: Cyanidin-3glucoside, Pt: Petunidin-3-glucoside, Pn: Peonidin-3-glucoside, Mv: Malvidin-3-glucoside, Dp-coum: Dp-3-O-(6-O-coumaroyl)-glucoside; Mv-caff: Mv-3-O-(6-O-caffeoyl)-glucoside; Pt-coum: Pt-3-O-(6-O-coumaroyl)-glucoside; Pn-coum: Pn-3-O-(6-O-

coumaroyl)-glucoside; Mv-coum: Mv-3-O-(6-O-coumaroyl)-glucoside (Table 3). Mv was the largest constituent, followed by Dp and Pn. In the mature fruit, NaCl treatment significantly increased the 5 non-acylated individual anthocyanins (Dp, Cy, Pt, Pn and Mv) (Table 3).

Total anthocyanin content (TAC) of grape skin extracts during ripening were shown in Fig. 1. The TAC during maturation were higher under NaCl treatments than that in control (Fig 1). According to the position of substituents on B-ring of flavonoid skeleton, five non-acylated individual anthocyanins can be divided into 3'- substituted anthocyanins (Cy, Pn) and 3'5'- substituted anthocyanins (Mv, Dp and Pt). During grape maturation, the account for the 3'5'- substituted anthocyanins (Mv, Dp and Pt) of the TAC increased from 87.17% to 88.31% in the control. After varasion, although NaCl treatment increased the content of Mv, Dp and Pt. The account for the 3'5'- substituted anthocyanins was lower than that in control. And the composition of 3'- substituted anthocyanins increased under salt treatment than control during maturation (Fig 1, Table 3).

Effects of NaCl treatment on enzyme activities

In the flavonoid pathway, the action of many related enzymes may contribute to the anthocyanin accumulation in grape skins (Duan et al., 2019). In order to better understand the impact of NaCl on anthocyanin biosynthesis, the activities of eight pivotal enzymes, CHS, CHI, DFR, F3H, F3'5'H, F3'H, LDOX and UFGT were investigated during grape ripening. The original step of flavonoid pathway is catalyzed by CHS to produce chalcone. During grape maturation, compared with the control, the 60 mM NaCl treated samples first had lower CHS activity levels at 90 DAA, then it significantly enhanced during ripening (Fig.2). At 120 DAA, the CHS activity of NaCl treated samples was significantly higher than the control group. CHI is one of the key enzymes in anthocyanin synthesis. It can catalyze isomerization of tetrahydroxychalcone. The CHI activity of all salt treatments increased rapidly after varasion. In the control group, the CHI decreased from 90 DAA to 120 DAA. Only 40 mM NaCl treated samples had higher CHI activity than control at 120 DAA (Fig.2). After CHS, F3H hydroxylates flavanones to form dihydroflavonols. Subsequently, DFR catalyses the conversion of dihydroflavonols to leucoanthocyanidins, followed by the production of anthocyanidin from leucoanthocyanidins, which involves LDOX (Boss et al., 1996). DFR is the key enzyme committed to anthocyanin and proanthocyanidin biosynthesis in the flavonoid biosynthetic pathway (Katsu et al., 2017). The DFR activity of the control group peaked at 105 DAA and then decreased. The NaCl treatments significantly increased the DFR activity in the ripen grape (Fig.2). F3H is part of the 2-oxoglutarate-dependent dioxygenases family and is fundamental for flavonol synthesis in Vitis. It is a crucial enzyme in regulating cyanidin derivatives (Kumar and Yadav, 2013). The F3H activity in the control increased rapidly after varasion. Only 100 mM NaCl treatment had higher F3H activity than control. F3'H promotes cyanidin and peonidin anthocyanin accumulations, while F3'5'H catalyzes the production of delphinidin and its derivatives petunidin and malvidin. These two enzymes competitively control di- and trihydroxylated anthocyanin synthesis (Falginella et al., 2010). The activities of F3'H and F3'5'H exhibited similar dynamic changes in the control groups. They decreased from 105 DAA to 120 DAA. However, the F3'5'H activity of each salt treatment was higher than that of the control. The 60mM NaCl treated samples first had lower F3'H activity levels, then it significantly increased throughout ripening. In mature grapes, the activity of F3'H under NaCl treatments was significantly greater than that of control (Fig.2).

LDOX can catalyze the transformation of colorless cyanidin and delphinidin into coloured anthocyanins. The LDOX activity in each salt treatment was significantly higher than that of the control. LDOX activity gradually increased with the salt concentration and peaked under 60 mM NaCl. But the 100 mM NaCl decreased LDOX activity from 105 DAA to 120 DAA during grape ripening (Fig.2). The enzyme UFGT catalyzes the final step of anthocyanin biosynthesis (Davies et al., 1997). The UFGT activity peaked at 105 DAA and then decreased during grape ripening. The 40 mM NaCl treated grapes got highest UFGT activity at 120 DAA, while UFGT of other salt treatments was lower than the control (Fig.2).

Correlation analysis between anthocyanin contents and key enzymes in anthocyanin biosynthesis

The correlation between the activity of eight enzymes and the total content of anthocyanins in grape skins during maturation was analyzed. The results showed that in the control group, only LDOX activity was positively correlated with total anthocyanin content ($p< 0.05$) (Table 4). After treatment with different concentrations of NaCl, only 100 mM treatment showed a significant positive correlation between LDOX activity and total anthocyanins ($P < 0.05$). And there was no significant correlation between the eight enzyme activities and total anthocyanins under other NaCl concentration (Table 4).

During anthocyanin synthesis, the enzymes F3'H and F3'5'H are responsible for the hydroxylation of the B-ring of flavonoids at position 3' or 5' (Falginella *et al*., 2010). In the test, the total trihydroxylated anthocyanins in the control group and NaCl treatment groups were correlated with F3'5'H, UFGT, IDOX activity and 100 mM NaCl treatment group, the total amount of trihydroxylated and dihydroxylated anthocyanins were positively correlated with LDOX activity (Table 5). However they were not significantly correlated with other enzyme activities (Table 5).

Discussion

Salinity can induce complex effects on grapevines. Moderate salt stress can improve the quality of plants. Low salt stress increased the content of total sugar and ascorbic acid in 'Moldova' grape fruit which improved the quality of grape (Gong *et al*., 2013). The organoleptic quality and flavours of table grapes are mostly determined by the content and composition of sugars and organic acids. Therefore, the ratio of TSS to TA is ordinarily used to assess fruit flavor. Tomatoes (Sato et al., 2006) and strawberries (Anna Keutgen, 2007) under moderate salinity have increased TSS, TA and stable TSS/TA amounts. Long-term NaCl treatment on Kyoho grape berry increased the average grain weight and cross diameter of grape fruit. Moderate salinity treatment raised the soluble sugars glucose, fructose and sucrose (Li et al., 2013). Our experiment indicates that different concentrations of NaCl decreased the average grain weight, the vertical and transverse diameter of grapes. And NaCl solution significantly increased the content of reducing sugar and TSS in mature grapes (Table 1). Glucose and fructose are the predominant sugars accumulated during berry ripening (Li et al., 2013). During grape ripening, the content of glucose and fructose in the fruits of different NaCl concentrations were significantly higher than that of the control (Table 2). The results are consistent with previous studies (Li et al., 2013; Parida et al., 2002; Sato et al., 2006). The altered soluble sugar content (Table 2) in grape fruit not only improve the sweetness and flavour under salinity, but also improve the potential alcohol of wine, which is conducive to the production of high-quality wine. Sugars can provide a framework for the biosynthesis of many secondary metabolites, such as phenols, terpenes and organic acids (Pan et al., 2009). The type and concentration of

organic acids in grape is an important aspect of quality control. Among the organic acids, tartaric acid and malic acid account for over 90% of the total acids. And tartaric acid is more sour than malic acid (Amerine et al., 1965; Liu et al., 2006). The altered percentage in the organic acid composition suggested a change in sourness under salinity. Although salt treatment reduced the yield of grape, it improved the fruit quality.

Flavonoids are reported to be responsive to various abiotic stresses (Treutter, 2007). Anthocyanins represent a group of natural flavonoid compounds in plants and are responsible for the coloration of berrys. The anthocyanin content of Kyoho grape berry increased after 20 mM and 60 mM NaCl treatment (Li et al., 2013). Salt stress increased the antioxidant capacity and anthocyanins in two strawberry cultivars (Keutgen and Pawelzik, 2008). Similar to the previous study, in our present work, NaCl treatment could enhance anthocyanin content at different development stages of grapes. When plants are under salt stress, they will spontaneously carry out a series of biochemical reactions to improve the adaptability to salt environment. The synthesis of compatible substances is one of the important strategies. The substances includes sugars, polyols, proline and flavonoids (Parida et al., 2002). Anthocyanins, as the main component of flavonoids, have the function of osmoregulation: the content of total anthocyanins in hypocotyls and cotyledons of tomato and purple cabbage seedlings cultivating with nutrient solutions of different salt concentrations is significantly higher than that of the control (Eryılmaz, 2006; Tahkokorpi et al., 2012). The nutrient solution containing 40 or 80 mmol/L NaCl enhanced the anthocyanin content of the salt resistant strawberry Korona. But the anthocyanin of the salt sensitive variety Elsanta decreased significantly (Keutgen and Pawelzik, 2007). Plants produce reactive oxygen and malondialdehyde (MDA) to cause cell damage under salt stress., As a natural non-enzyme antioxidant, anthocyanin will react with superoxide dismutase (SOD) in plants to eliminate excessive active oxygen radicals and reduce the damage of MDA to cell membrane, which will improve the adaptability of plants to salt environment (Keutgen and Pawelzik, 2007). Therefore, one reason of the elevated content of anthocyanin could be attributed to its salt resistance in plants.

Anthocyanins are secondary metabolites, primarily synthesized via phenylpropanoid and flavonoid pathways. These pathways consist of a number of enzymatic steps, each of which catalyzed by a consecutive reaction for anthocyanin biosynthesis (Zorenc et al., 2017). In the study, we explored the activity of key regulatory enzymes in the flavonoid pathway of anthocyanin metabolism in response to NaCl treatment. The activity of eight enzymes during grape riping were shown in Fig. 2. Only LDOX activity was positively correlated with total anthocyanin content ($p < 0.05$) in the control and 100 mM NaCl treated group. And there was no significant correlation between the eight enzyme activities and total anthocyanins under other NaCl concentration (Fig. 2). We also detected no significant correlation between di- and trihydroxylated anthocyanin with F3’H and F3’5’H activity. Flavonoids mainly include flavanone, anthocyanin and proanthocyanidins. They share a part of synthesis path in grape skins (Matus et al., 2009). The nonsignificant difference of correlation may be because salt stress also affects the activity of enzymes related to the non anthocyanin flavonoids synthesis, or the expression level of enzyme genes. Therefore anthocyanin precursors are more used in the synthesis of other flavonoids.

The regulatory effect of *MYB* transcription factor on flavonoids has been confirmed in many plants (Feng et al., 2010; Niu et al., 2010; Umemura et al., 2013). *MYB* regulating flavonoid synthesis has been shown to be differentially affected by abiotic stimuli in grapevines (Matus et al., 2010). *MYB1* is specifically expressed in grape skin and induce anthocyanidin synthesis. *MYB2* can activate the promoter of *UFGT* efficiently (Guohui et al., 2013). Therefore, the anthocyanin content in the test is not only the result of the co regulation of regulatory genes and structural genes, but also closely related to the activity of related enzymes or the expression of coding genes in the synthesis of other flavonoids.

The synthesis and decomposition of anthocyanins keep a dynamic balance in grape skins, so the total anthocyanins in the experiment are net content. Various physical and chemical factors will destroy anthocyanin’s stability and make it degrade *in vitro*. The degradation of anthocyanin *in vitro* has been widely studied, but there are few studies on degradation *in vivo*. Anthocyanins are secondary metabolites of plants in response to environmental changes. Salt stress not only affect the biosynthesis genes and enzymes, but also affect the enzymes or genes in the pathway of anthocyanin catabolism. Both anabolism and catabolism determine the change of anthocyanin content and composition in plants. Further studies are needed in catabolic enzymes and genes on anthocyanin synthesis.

Conclusion

In summary, the application of NaCl to grape foliage effectively increased the quality of the Cabernet Sauvignon grape, improving soluble sugar, organic acid, phenolics and tannin contents. Salt treatment significantly increased the total anthocyanin contents in grape skins after varasion. And 40mm NaCl has the greatest impact on the anthocyanin content. However, because the anthocyanin synthesis of grapes are influenced by many environmental factors in field, such as light, temperature, rainfall and biotic stress. Furthermore, more researches are needed into the molecular mechanisms of exogenous NaCl increasing the anthocyanin content and synthesis of grape skins.

Abbreviations

bHLH
b helix-loop-helix
CHS
Chalcone synthase
Cy
Cyanidin-3glucoside,
DAA
Days after anthesis
DFR
Flavonol-4-reductase
DNS

Dinitrosalicylic acid
Dp
Delphinidin-3-glucoside,
Dp-coum
Dp-3-O-(6-O-coumaroyl)-glucoside;
ELISA
Enzyme linked immunosorbent assay
F3'5'H
Flavonoid 3',5'-hydroxylase
F3H
Flavanone 3-hydroxylase
F3'H
Flavonoid 3'-hydroxylase
HPLC
High Performance Liquid Chromatography
LDOX
Leucoanthocyanidin dioxygenase
MDA
Malondialdehyde
Mv
Malvidin-3-glucoside,
Mv-caff
Mv-3-O-(6-O-caffeoyl)-glucoside;
Mv-coum
Mv-3-O-(6-O-coumaroyl)-glucoside
PAL
Phenylalanine ammonia-lyase
Pn
Peonidin-3-glucoside,
Pn-coum
Pn-3-O-(6-O-coumaroyl)-glucoside;
Pt
Petunidin-3-glucoside,
Pt-coum
Pt-3-O-(6-O-coumaroyl)-glucoside;
SOD
Superoxide dismutase
TA
Titratable acid
TAC
Total anthocyanin content
TSS
Total soluble solids
UFGT
Flavonoids 3-O-glucosyltransferase
UPLC
Ultra performance liquid chromatography

Declarations

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Authors' contributions

XY designed the research. XY, and XT performed the experiments and collected the data. ZY and XZ analyzed the data and XY wrote the manuscript. NH edited the manuscript and provided guidance during experimentation. All authors read and approved the fnal manuscript.

Availability of data and materials

All of the data and materials are available upon request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interest

The authors declare that they have no competing interests.

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Tables

Table 1 Changes in fruit quality parameters of the C. Sauvignon grape under salinity stress.

Quality parameters	NaCl concentration (mM)			
	0	40	60	100
Berry fresh mass (g)	1.67±0.01a	1.45±0.02c	1.52±0.01b	1.49±0.03b
Vertical diameter (mm)	14.16±0.88a	13.31±0.85b	13.79±0.86ab	13.46±0.93b
Transverse diameter (mm)	14.10±0.98a	13.30±0.82b	13.33±0.93b	13.21±0.91c
Total Solube solids (°Brix)	17.33±0.29c	20.17±0.29a	19.67±0.29a	20.00±0.00a
Reducing sugar (mg/g)	184.05±2.75d	237.75±1.14a	229.39±2.12b	231.37±1.37b
Titratable acidity (g /L)	7.10±0.10a	6.00±0.03c	5.66±0.07d	7.10±0.04a
Phenols (mg/g)	6.33±0.25b	6.56±0.47b	7.52±0.38a	7.73±0.63a
Tannin (mg/g)	6.24±0.16b	6.54±0.23b	7.84±0.25a	7.84±0.42a

Values are reported as the mean ± SD. Different letters indicate significant differences at $p < 0.05$

Table 2 Modification of soluble sugars and organic acids in the C. Sauvignon berrys in response to NaCl.

Soluble sugars and organic acids (mg/g)	NaCl concentration (mM)			
	0	40	60	100
Glucose	76.16±1.48c	85.06±0.80a	79.14±0.72b	84.27±1.77a
Fructose	73.49±0.72c	84.08±1.29a	78.24±2.59b	82.29±2.36a
Sum of soluble sugars	149.64±2.20c	169.13±1.25a	157.39±2.83b	166.56±3.77a
Tartaric acid	11.64±1.25b (46.62%)	11.34±1.01b (50%)	12.11±2.05ab (49.37%)	14.20±0.86ab (49.62%)
Malic acid	12.00±2.02a (48.06%)	10.14±1.72a (44.70%)	11.00±2.83a (44.84%)	12.72±0.09a (44.44%)
Citric acid	0.71±0.01ab (2.84%)	0.61±0.08b (2.69%)	0.72±0.16ab (2.94%)	0.79±0.01ab (2.76%)
Oxalic acid	0.63±0.10b (2.52%)	0.59±0.07b (2.60%)	0.70±0.14b (2.85%)	0.91±0.06a (3.18%)
Sum of organic acids	24.97±3.37a	22.68±2.87a	24.53±5.17a	28.62±0.88a

Values are reported as the mean ± SD. Different letters indicate significant differences at $p < 0.05$. Number in bracket indicates the percentage of the corresponding composition.

Table 3 Effects of different NaCl concentrations on the contents and compositions of anthocyanin

DAA	NaCl (mM)	Delphinidin-3-glucoside	Cyaniding-3-glucoside	Petunidin-3-glucoside	Peonidin-3-glucoside	Malvidin-3-glucoside	Dp-3-O-(6-O-coumaroyl)-glucoside	Mv-3-O-(6-O-caffeoyl)-glucoside	Pt-3-O-(6-coumaroy glucoside
90	0	506.96±5.48a	95.55±0.88a	368.70±5.06a	489.70±5.79a	2282.12±51.05a	16.27±2.84a	14.56±2.22a	17.63±1.8
	40	155.98±4.54e	37.92±0.14d	142.27±1.49d	322.25±1.46d	2008.85±17.20d	10.60±4.70ab	14.81±1.15a	10.31±1.1
	60	331.96±11.97b	62.34±1.96b	262.07±5.60b	461.68±12.93b	3031.77±70.65b	14.44±1.19a	13.66±1.75a	17.50±0.8
	100	232.32±2.94c	58.04±1.36c	190.46±2.36c	349.18±7.22c	2215.56±34.53c	10.17±0.73ab	14.80±1.43a	12.89±1.5
105	0	434.63±11.67c	70.40±2.38d	336.42±5.79c	488.23±6.44c	3342.32±49.64c	12.10±3.52b	17.50±0.67a	15.40±2.1
	40	483.03±21.62b	102.65±4.56b	390.13±13.03b	660.60±9.53a	3895.65±66.94b	12.16±1.80b	14.50±1.52a	14.12±0.5
	60	496.07±15.26b	87.13±1.04c	409.54±11.78a	638.16±17.37a	4355.92±121.24a	13.83±1.61b	15.32±2.10a	16.60±2.4
	100	547.79±5.23a	106.61±0.60ab	411.89±1.94a	642.07±3.34a	3849.05±28.68b	19.79±0.53a	16.56±1.25a	20.80±0.1
120	0	268.24±2.84e	46.47±0.48d	233.39±5.21d	391.57±16.47d	3028.66±43.63d	9.04±3.24b	14.41±2.95ab	13.54±3.5
	40	368.38±9.93b	76.33±2.29b	307.50±8.28b	529.18±11.75b	3607.39±42.50a	15.66±2.27a	17.27±1.13a	19.64±1.3
	60	351.05±7.71c	62.10±1.94c	278.75±3.62c	453.42±8.09c	3149.22±47.28c	9.90±2.27b	12.84±1.73b	12.39±3.2
	100	472.35±8.64a	103.35±1.72a	357.33±4.59a	566.36±8.25a	3452.03±21.20b	15.87±0.71a	13.02±0.51b	18.20±0.9

Values are reported as the mean ± SD. Different letters indicate significant differences at $p < 0.05$.

Table 4 Correlation between enzyme activity and anthocyanin contents under NaCl treatment

Treatments	CHS	CHI	DFR	F3H	F3'5'H	F3'H	UGFT	LDOX
CK	0.823	0.216	0.553	-0.685	0.689	0.981	0.239	**0.999***
40 mM NaCl	0.9	0.869	-0.368	-0.672	0.378	-0.667	0.933	0.544
60 mM NaCl	0.067	0.549	-0.9	-0.665	-0.345	0.568	-0.948	0.066
100 mM NaCl	0.993	0.471	0.111	-0.645	0.913	0.869	0.921	**0.999***
Note: * indicates significant differences at $p < 0.05$.								

Table 5 Correlation between enzyme activity and di/trihydroxylated anthocyanin content

Anthocyanin	Enzyme	NaCl concentration (mM)			
		0	40	60	100
Trihydroxylated anthocyanin	F3'5'H	0.677	0.355	-0.366	0.914
	UFGT	0.255	0.941	-0.955	0.921
	LDOX	**0.998***	0.565	0.089	**0.998***
	F3'5'H/UFGT	0.524	-0.407	0.97	0.937
Dihydroxylated anthocyanin	F3'H	0.993	-0.778	0.413	0.874
	UFGT	0.166	0.863	-0.876	0.917
	LDOX	**1.000***	0.403	-0.112	**0.999***
	F3'H/UFGT	0.884	-0.992	0.854	0.678

Note: * indicates significant differences at $p < 0.05$.

Figures

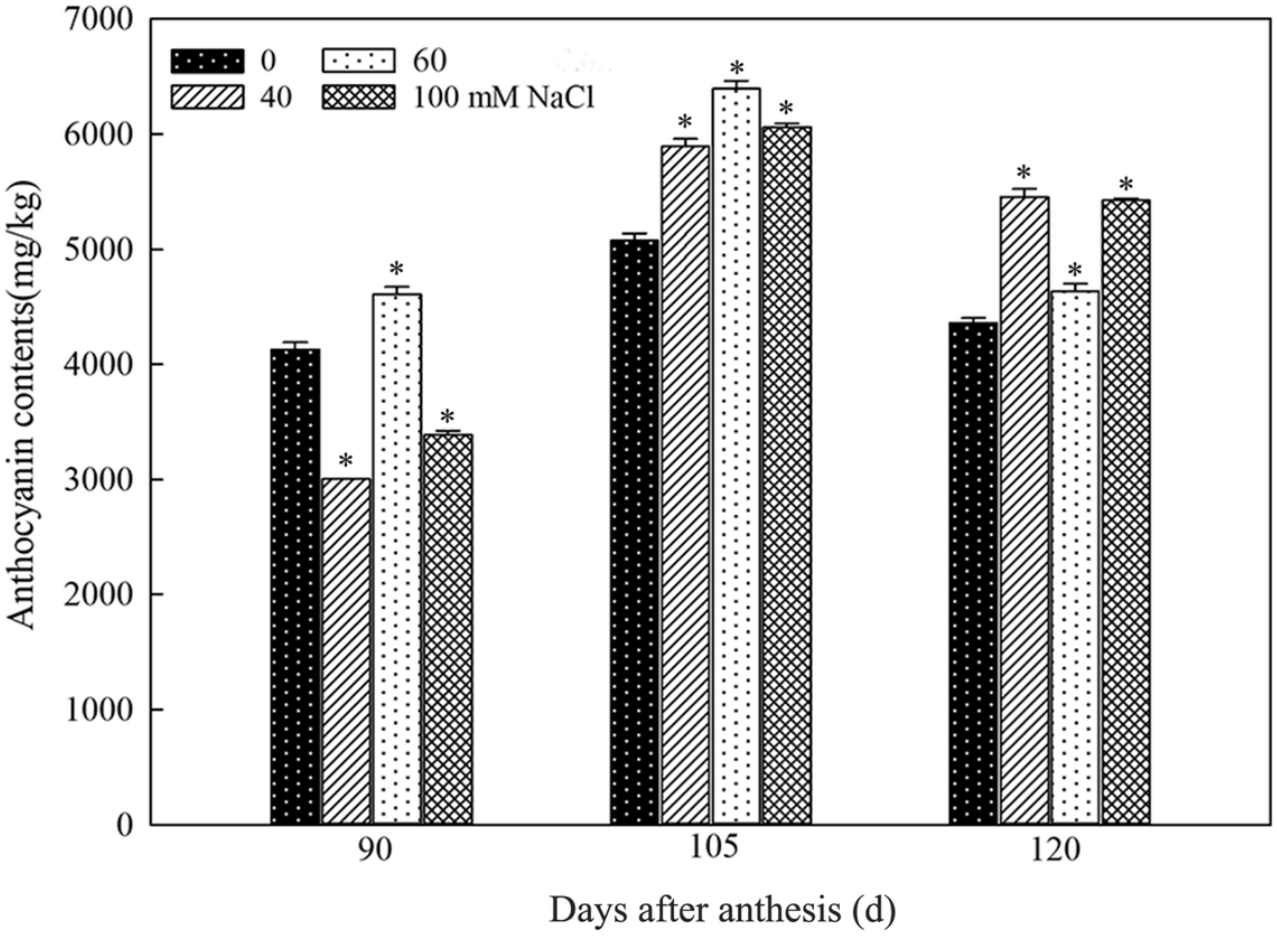

Figure 1

Effect of NaCl treatment on total anthocyanin contents of C. Sauvignon berrys during berry riping. Asterisk indicates statistical significance at $P < 0.05$. Data are means ± SD of three biological replicates.

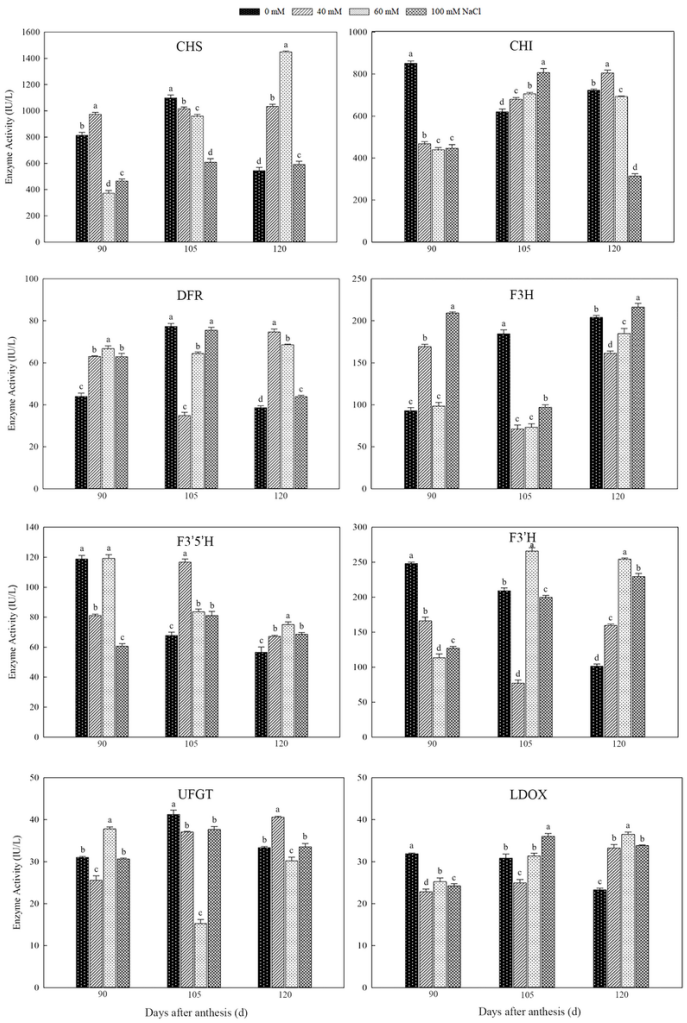

Figure 2

The activity of CHS, CHI, DFR, F3H, F3’5’H, F3’H, UFGT and LDOX in grapes under NaCl treatment during riping.