Research Article

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Changes in sugar content and related enzyme activities in table grape (*Vitis vinifera* L.) in response to foliar selenium fertilizer

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Abstract

BACKGROUND: Spraying selenium (Se) fertilizer is an effective method for Se-enriched fruit production. Sugar content in fruit is the major factor determining berry quality. However, changes in sugar metabolism in response to Se fertilizer are unclear. Hence, this study was conducted to identify the effects of Se fertilizer on sugar metabolism and related enzyme activities of grape berries. Additionally, production of leaves with and without Se fertilizer was also investigated.

RESULTS: Acid invertase (AI) activity, total soluble sugar and Se content in berries, and photosynthetic rate in leaves produced under Se fertilizer treatments were higher than that of control. Glucose and fructose were the primary sugars in berries, with a trace of sucrose. In both berries and leaves, neutral invertase activity was lower than AI, there was no significant difference in neutral invertase, sucrose synthase and sucrose phosphate synthase between Se fertilizer-treated and control. In berries, AI showed a significant positive correlation with glucose and fructose; also Se content was significantly correlated with sugar content.

CONCLUSION: AI played an important role in the process of sugar accumulation in berries; high AI activity in berries and photosynthetic rate in leaves could explain the mechanism by which Se fertilizer affected sugar accumulation in berries.

Supporting information may be found in the online version of this article.

Keywords: Hutai No. 08; sugar components; metabolic enzymes; dynamic change

INTRODUCTION

Sugar accumulation is an important event in the berry-ripening physiology of grapevine, and is essential for superior enological characteristics and product value.[1] Also sugar content in grape berries is the major factor determining fruit quality. Sugar is synthesized in leaves as a result of photosynthesis and imported via the phloem into the berry in the form of sucrose.[2] After unloading of sucrose in the berry, sucrose is hydrolysed; meanwhile glucose and fructose accumulate.[3] The hydrolysis is related to sucrose metabolic enzymes. Sugar accumulation in the berry depends on the activity of sugar-metabolizing enzymes during ripening. The enzymes that regulate sugar accumulation and metabolism in grape berries include invertase, sucrose synthase (SS) and sucrose phosphate synthase (SPS).[4] Invertases can be classified according to their ideal pH of activity to acid invertases (AI) and neutral invertases (NI).[5]

Selenium (Se) is an essential trace element in human nutrition,[6] but Se deficiency is still a serious nutritional and health problem worldwide. Spraying Se fertilizer is a new practice for high-quality production in tree fruit cultivation, and an effective method of developing Se-enriched fruit.[7] Various studies have revealed that Se fertilizer can improve yield and quality in wheat and rice.[8,9] Moreover, it can effectively increase Se content of crop edible parts[10,11] and reduce accumulation of heavy metals.[12] Most of those studies, however, have selected inorganic Se as the main source of Se fertilizer, and more focused on the effects of application method,[9] application rate and number on crop quality and Se concentration.[13,14]

Although studies have reported Se fertilizer can both induce an increase in leaf photosynthetic rate and improve fruit quality, no explanation for this phenomenon was given. In addition, study of foliar organic Se fertilizer on accumulation of sugars within the berry dynamics of closely related enzyme activities has not been reported in detail. The objectives of this study were to examine the effects of spraying organic Se fertilizer on sugar metabolism and related enzyme activities of grape berries during grape development, explore the mechanism of exogenous organic Se on

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sugar metabolic effects in grape berries and, furthermore, to provide scientific guidance for Se fertilizer application in high-quality Se-enriched grape production.

MATERIALS AND METHODS

Experiment design

The experiment was carried out in the experimental greenhouse of the Institute of Soil and Water Conservation, Northwest A&F University, in Yangling (34° 12′ to 34° 20′ N, 108° to 108° 7′ E; elevation 560 m), Shaanxi, China, from February to September 2016. The annual mean air temperature was 12.9 °C and the average annual precipitation was about 635.1 mm. The soil texture was dark loessial soil (46.4% sand, 37.0% silt and 16.6% clay, on average) with a pH value of 7.9. Soil water-holding capacity was 24% (mass basis) and the soil bulk density was 1.2 g cm^{-3}; soil electrical conductivity was 0.7 mS cm^{-1}; Se content of soil was 59 μg kg^{-1}; organic matter content was 14.66 g kg^{-1}; total N content was 0.82 g kg^{-1}; total P_2O_5 content was 0.99 g kg^{-1}; available N (1 mol L^{-1} NaOH hydrolysis) was 28.75 mg kg^{-1}; available P (0.5 mol L^{-1} $NaHCO_3$) was 30.46 mg kg^{-1}; and available K (1 mol L^{-1} neutral NH_4OAc) was 153.68 mg kg^{-1}.

The experiment was performed on Hutai No. 08 variety, which is a European and American species, including control (CK, pure water) and Se fertilizer treatment (SE; amino acid-chelated Se-enriched foliar fertilizer provided by Shaanxi Yangling Macao Bond Biological Science Co. Ltd. It contained Se, Ti and a variety of chelating trace elements, Cu + Fe + Mn + Zn + B ≥ 100 g L^{-1}, organic Se ≥ 60 g L^{-1}). Se fertilizer, i.e. the aforesaid mixture, which was diluted 500 times with pure water, was applied to the whole canopy as a foliar spray when the berries were at 19, 30 and 40 days after fruit set during the evening hours (17:00–18:00) on 17, 28 May and 8 June 2016, respectively. Similarly, pure water was applied to control vines. The experiment was conducted on eight plots (6.5 m × 3.5 m), composed of 16 vines of the stated variety at the age of 3 years. The experimental design was a randomized complete block design. The plantation was managed following the usual local procedures; organic fertilizer was applied at 180 g per tree after pruning in 2015; and K fertilizer and urea were applied at doses of 40 g and 20 g per tree, respectively, on 12 May 2016. The plots were irrigated after each fertilization. In addition, three supplementary irrigations were applied at fruit set, fruit swelling and veraison period, respectively, according to 90% of field capacity of farmland irrigation, when soil water content was <60% of field capacity using time domain reflectometry (TDR) measurement. Each vine reserved seven to nine clusters fruit, and kept 40–60 fruits per cluster when fruit thinning.

Sample measurement

Six samples of Hutai No. 08 were collected on 23 June, 4, 14, 25 July, and 6 and 30 August 2016, respectively. First, choosing fruit trees such that total fruit number was approximate, with five clusters gathered randomly in the same position of each treatment to avoid fruit position effect, 20 fruits were selected randomly in the middle of each cluster for every sampling, so the total sample number of each treatment was 100 fruits. Berry samples were rinsed with ice-distilled water three times and surface water removed; berries were then peeled and, after removal of seeds, fast ground using a chilled mortar and pestle, then placed in a glass bottle at 4 °C pending analysis. Each analysis was repeated three times.

Grape berry growth and development

Weights of 20 berries in each group were measured on every sampling with a Milli analytical balance, then individual berry weight was calculated. Fruit redness was measured using a portable handheld colorimeter (Konika-Minolta Chroma Meter CR-400, Minolta Corporation, Ltd, Osaka, Japan); from each treatment five fruits were selected, and measurements of a^* value were made at the equatorial plane of each fruit [a^* green ($-a^*$) to red ($+a^*$)].[15] Total soluble solids (TSS; °Brix) was determined using an ATAGO PR-101 digital refractometer[16] and titratable acidity (TA) by titration with 0.1 mol L^{-1} NaOH.

Sugar determination

Total soluble sugar was measured by the phenol–sulfuric acid method. Fructose and sucrose were measured by resorcinol spectrophotometry, and the enzyme preparation method was used to measure glucose content.[17]

Enzyme extraction and assays

Sugar metabolism enzymes were extracted using methods similar to Keller and Ludlow.[18] Fruit or leaf samples (1–2 g) were ground in a chilled mortar with a total of 8 mL buffer three times for 3–5 min. The buffer contained 50 mmol L^{-1} HEPES–NaOH (pH 7.5), 10 mmol L^{-1} $MgCl_2$, 1 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA), 2.5 mmol L^{-1} dithiothreitol, 0.05% (v/v) Triton X-100 and 0.1% (w/v) bovine serum albumin. Homogenates were centrifuged (4 °C at 15 000 × g for 10 min). Supernatants were desalted at 2 °C immediately by buffer (no Triton X-100) diluted 10 times for 24 h. Ensure the enzyme extract after dialysis was constant volume of 10 mL for assay using. All enzymes were extracted and desalted at 0–4 °C.

Sucrose synthase and sucrose phosphate synthaseassays[19]

Reaction mixture (100 μL) to determine SS activity contained 100 mmol L^{-1} phosphate buffer (pH 8.0), 15 mmol L^{-1} $MgCl_2$, 4 mmol L^{-1} uridine diphosphate glucose (UDPG), 60 mmol L^{-1} fructose, 1 mmol L^{-1} EDTA and 50 μL desalted extract, then distilled water was added to 0.4 mL. The mixture was incubated at 34 °C and terminated at 60 min with 0.2 mL of 2 mol L^{-1} NaOH. Tubes were placed in boiling water for 10 min to destroy any unreacted fructose or fructose 6-phosphate. After cooling, 2.8 mL of 30% hydrochloric acid and 0.8 mL of 0.1% resorcinol were added and incubated in a 80 °C water bath for 10 min. After cooling, color development was measured at 480 nm. The blank tube contained 50 μL distilled water; the comparison tube contained 50 μL inactivated enzyme extract that put enzyme extract in a boiling water bath for 10 min, and similarly for the rest of procedure. The difference can be used to calculate SS activity. The procedure for the SPS assay was identical to that of SS except the reaction mixtures contained 100 mmol L^{-1} borate buffer (pH 8.0), 15 mmol L^{-1} $MgCl_2$, 5 mmol L^{-1} fructose 6-phosphate, 15 mmol L^{-1} glucose 6-phosphate, 10 mmol L^{-1} UDPG and 1 mmol L^{-1} EDTA. Reaction mixtures were incubated at 34 °C for 30 min.

Acid invertase and neutral invertase assays[20]

Reaction mixtures (1 mL) to determine AI activity contained 100 mmol L^{-1} NaAc-HAc buffer solution (pH 4.8), 100 mmol L^{-1} sucrose and 0.1 mL desalted extract. Reaction mixtures were incubated for 20 min at 37 °C and terminated by placing tubes in boiling water for 5 min. After cooling, 1 mL 3,5-dinitrosalicylic acid was added and the mixture was incubated in a boiling water bath for

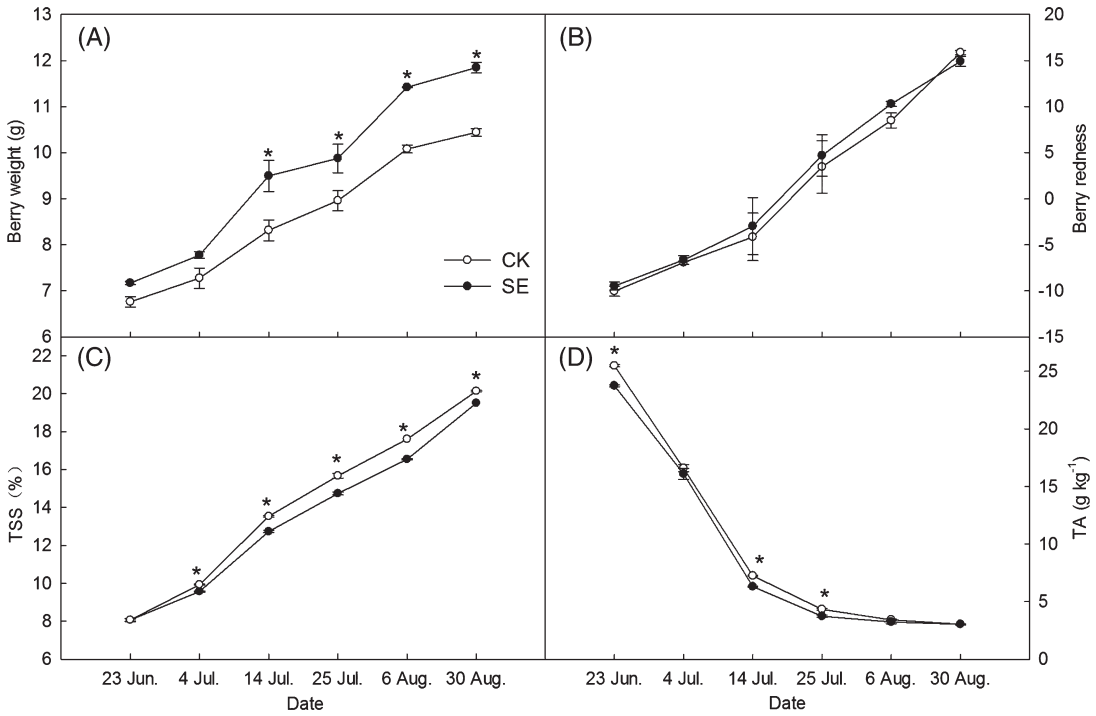

Figure 1. Changes in berry weight (A), berry redness (from green (−) to red (+)) (B), total soluble solids (TSS) (C), and titratable acidity (TA) (D) for control (CK) and Se fertilizer treatment (SE) during berry growth and development. Vertical bars represent standard error. Asterisk shows significant differences (*$P < 0.05$) between CK and SE groups.

5 min. After cooling, color development was measured at 540 nm. In addition, 100 μL distilled water and the 100 μL inactivated enzyme extract was taken as the black and comparison, and similarly for the rest of procedure. The difference can be used to calculate AI activity. Reaction mixtures (1 mL) for determination of NI contained 100 mmol L^{-1} phosphate buffer (pH 7.5), 100 mmol L^{-1} sucrose and 0.1 mL desalted extract. The assay and hexose determination was otherwise the same as for AI.

Determination of photosynthetic rate

The day before sampling, photosynthesis of leaves in corresponding clusters leaves was measured using a LICOR-6400 portable photosynthesis system; also the chlorophyll content of the leaves was measured using a SPAD-502 portable chlorophyll meter.

Determination of selenium content

The content of Se was determined by inductively coupled plasma mass spectrometry.[21]

Statistical analysis

The data were analyzed with version 20.0 of SPSS (Statistica, Tulsa, OK, USA). Statistically significant differences ($P < 0.05$) from different treatments were revealed after one-way analysis of variance (ANOVA), and multiple comparison followed by Duncan's test. Graphs were plotted using SigmaPlot 12.5.

RESULTS

Changes in berry weight, peel color, total soluble solids and titratable acidity

Grape berry growth measured as changes in berry weight exhibited a continuous increase in growth pattern, and berry color changed from green to red for both CK and SE clusters (Fig. 1A, B). During the whole growth period, there was no significant difference in berry redness between the CK and SE clusters (Fig. 1B). However, the berry weight in SE was significantly greater than that of CK after 14 July (Fig. 1A). Se fertilizer resulted in increased berry weight but had no effect on fruit color.

The patterns of change in TSS and TA content were similar both in the SE and CK groups, and the pattern showed a continuous increase in TSS and continuous decline in TA concentration (Fig. 1C, D). TSS content increased concurrently with the decline in TA content. However, TSS content in the SE group was significantly greater than that of CK except for 23 June (Fig. 1C). TA content in berries of both treatments declined rapidly before fruit coloring. TA content in berries under SE was significantly lower than in CK berries for most of the berry development, but there was no significant difference at harvest (Fig. 1D).

Selenium content in grape berries

With the growth of fruit, Se content in berries increased constantly for both SE and CK groups, and there was a significant difference between Se content in berries of SE and that of CK ($P < 0.05$). Se content in treated berries was 22.90 μg kg^{-1} at maturity; the raised rate was 33.56% compared to that of CK, meet the standard of the Se-enriched fruits (20–100 ug kg^{-1}). In addition, correlation analysis showed that Se content in berries showed a significant positive correlation with sugar (total soluble sugar, sucrose, glucose and fructose) content for CK; meanwhile, there was a significant positive correlation with sugar content except in the case of glucose for SE (Table 1).

The dynamic changes in sugar content of grape berries and leaves

During fruit development, change in fructose, glucose and total soluble sugar content showed a similar pattern in fruit from

Table 1. Correlation analysis of sugar and Se content in grape berries in Se fertilizer-treated and control grapevines.

Relationship	Control	Se fertilizer
Total soluble sugar–Se	0.863*	0.873*
Sucrose–Se	0.993**	0.990**
Glucose–Se	0.815*	0.808
Fructose–Se	0.882*	0.883*

Asterisks indicate that correlation is significant at the **0.01 and *0.05 level.

both CK and SE groups (Fig. 3A, C, D). The concentration of fructose, glucose and total sugar increased rapidly in the early coloring phase and then increased slowly, reaching a peak at maturity. The peak concentration of glucose and fructose in grape berries from the CK plants was 60.18 and 60.89 g kg^{-1} fresh weight (FW), whereas the concentration in SE berries was 63.24 and 65.40 g kg^{-1} FW, which was significantly higher than in CK (Fig. 3C, D). There were small quantities of sucrose compared to glucose and fructose content in the grape berries (Fig. 3B). Very little sucrose accumulated at the early stage, the minimum value being only 1.04 g kg^{-1} FW, but it then accumulated quickly from 14 July, reached 8.60 g kg^{-1} FW at maturity. During the quick sucrose accumulation, the sucrose content in fruit in the SE group was significantly higher than that of CK (Fig. 3B). The total soluble sugar content was determined mainly by glucose and fructose content. The concentration of glucose and fructose approximately maintained a 1:1 ratio. Total soluble sugar content in grape berries showed a significant difference between SE and CK groups ($P < 0.05$) (Fig. 3A). Thus, regarding accumulation of different sugar components in grape berries, glucose and fructose occurred earlier than sucrose over the whole growth period.

With growth of grape berries, the sucrose content in leaves gradually rose, reaching its highest level on 25 July, then falling until the end of August, because the leaves senesce and photosynthetic ability abate (Fig. 4B). Variance analysis results showed that sucrose content in leaves in the SE group was significant higher than the CK except for 23 June ($P < 0.05$). Change in fructose and glucose content showed a similar pattern in leaves from both CK and SE groups (Fig. 4C, D). During early growth, glucose content was slightly higher than fructose, before falling, ranging from 4.61 to 12.43 g kg^{-1} FW for glucose and fructose content, respectively. Content of glucose and fructose in leaves was obviously low on 4 and 14 July, possibly due to cloudy weather, affecting the leaves' ability to photosynthesize, leading to a lower photosynthetic product. Variance analysis results showed that the content of fructose and glucose in leaves in the SE group was significantly higher than CK after 14 July ($P < 0.05$). The total soluble sugar content in leaves was similar to glucose and fructose content, but it was reduced after 25 July for both CK and SE groups, which may also be because of leaf senescence (Fig. 4A).

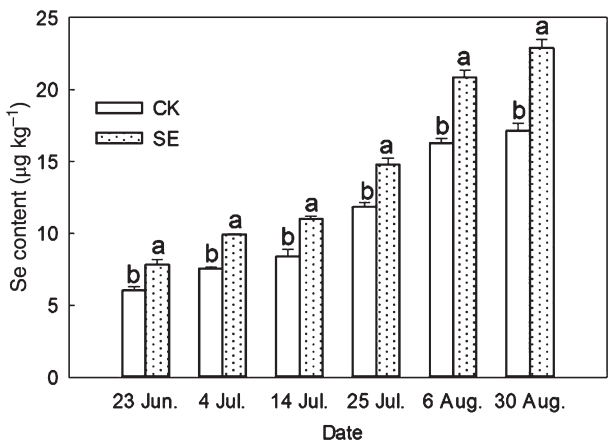

Figure 2. Changes in Se content for control (CK) and Se fertilizer treatment (SE) during berry growth and development. Different letters above columns show significant differences ($P < 0.05$) between CK and SE groups.

The dynamic changes of enzyme activities in grape berries and leaves

Enzyme activity in grape berries

In the grape berry growth period, AI activity in berries increased and peaked on 25 July before decreasing, but activity was still at a high level (Fig. 5A). Activity of AI in berries from the SE group was significantly higher than that of CK during all stages of berry development ($P < 0.05$). Dynamic change in NI showed a similar pattern in fruit during all stages of berry development for both treatments; it increased and peaked on 14 July, then decreased (Fig. 5B). However, NI activity in berries was lower than AI activity for both treatments. The NI enzyme activity in fruit from SE grapevines was also higher than that of control, but there the difference was not significant ($P < 0.05$) (Fig. 5B).

SS activity presented fluctuating changes during fruit development, peaking on 4 and 25 July for both treatments (Fig. 5C). The SS activity ranged from 12.9 to 28.24 μg sucrose g^{-1} FW min^{-1}, and there was no significant difference in SS activity in fruit from SE and CK groups ($P < 0.05$). Activity of SPS increased during berry development, peaking on 14 July with 27.33 μg sucrose g^{-1} FW min^{-1}, then falling to a minimum at 14.72 μg sucrose g^{-1} FW min^{-1} at maturity for both CK and SE groups (Fig. 5D). Variance analysis showed that Se fertilizer had no significant effect on SPS activity ($P < 0.05$).

Enzyme activity in leaves

AI activity in grape leaves increased first and dropped to a minimum on 14 July, then increased until fruit ripening (Fig. 6A). There was no significant difference between SE and CK groups with reference to AI activity ($P < 0.05$). NI activity in grape leaves was lower than AI activity for both SE and CK groups, and changes in volatility ranged from 11.09 to 18.16 μg glucose g^{-1} FW min^{-1} (Fig. 6B). Variance analysis showed that there was no significant difference between SE and CK groups ($P < 0.05$). AI and NI in leaves were all lower than that in fruits.

SS activity in leaves present fluctuating changes for both treatments; it peaked at 31.76 μg sucrose g^{-1} FW min^{-1} and troughed at 11.95 μg sucrose g^{-1} FW min^{-1} on 4 July and 6 August, respectively (Fig. 6C). There was no significant difference between SE and CK groups for SS activity in leaves ($P < 0.05$). SPS activity in leaves increased first and then decreased to a minimum on 25 July; it then increased again until fruit ripening, reaching a maximum at 18.33 μg sucrose g^{-1} FW min^{-1} at maturity (Fig. 6D). There was no significant difference between SE and CK groups for SPS activity in leaves ($P < 0.05$).

Chlorophyll content and photosynthetic rate in leaves

Changes in chlorophyll content in leaves increased first, then decreased to a minimum until maturity for both CK and SE groups.

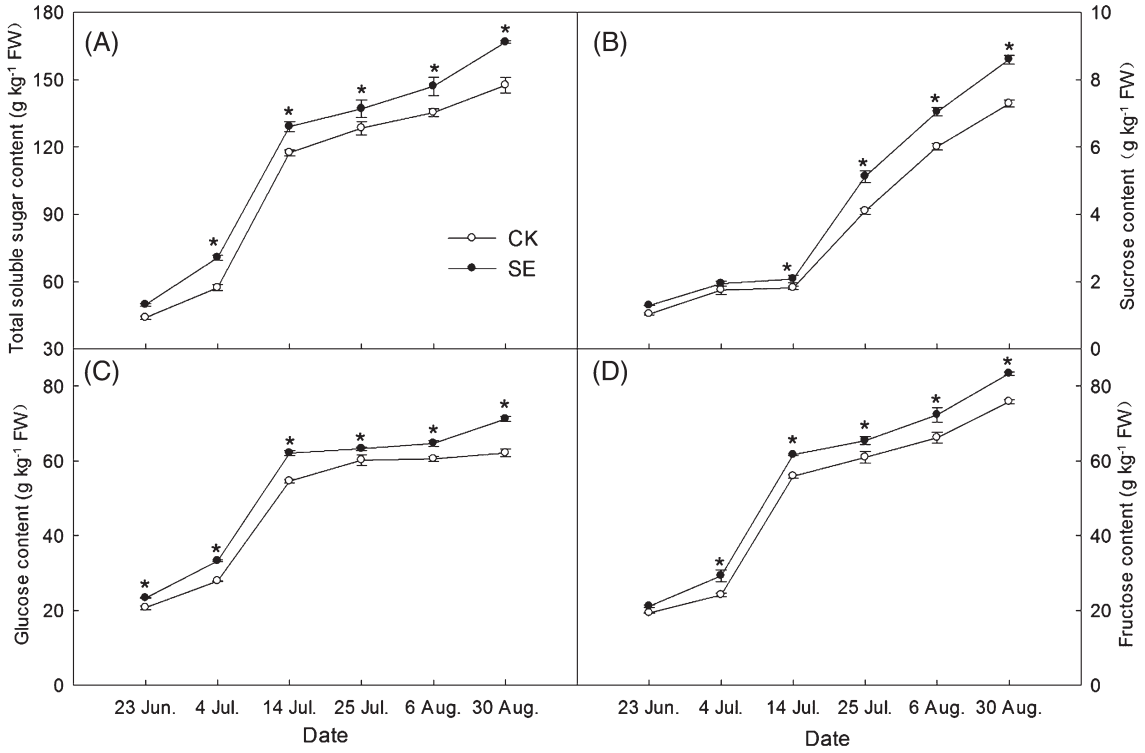

Figure 3. Developmental changes in sucrose, glucose, fructose and total soluble sugar content for control (CK) and Se fertilizer treatment (SE) in grape berries during berry growth and development. Vertical bars represent standard error. Asterisk shows significant differences (*$P < 0.05$) between CK and SE groups.

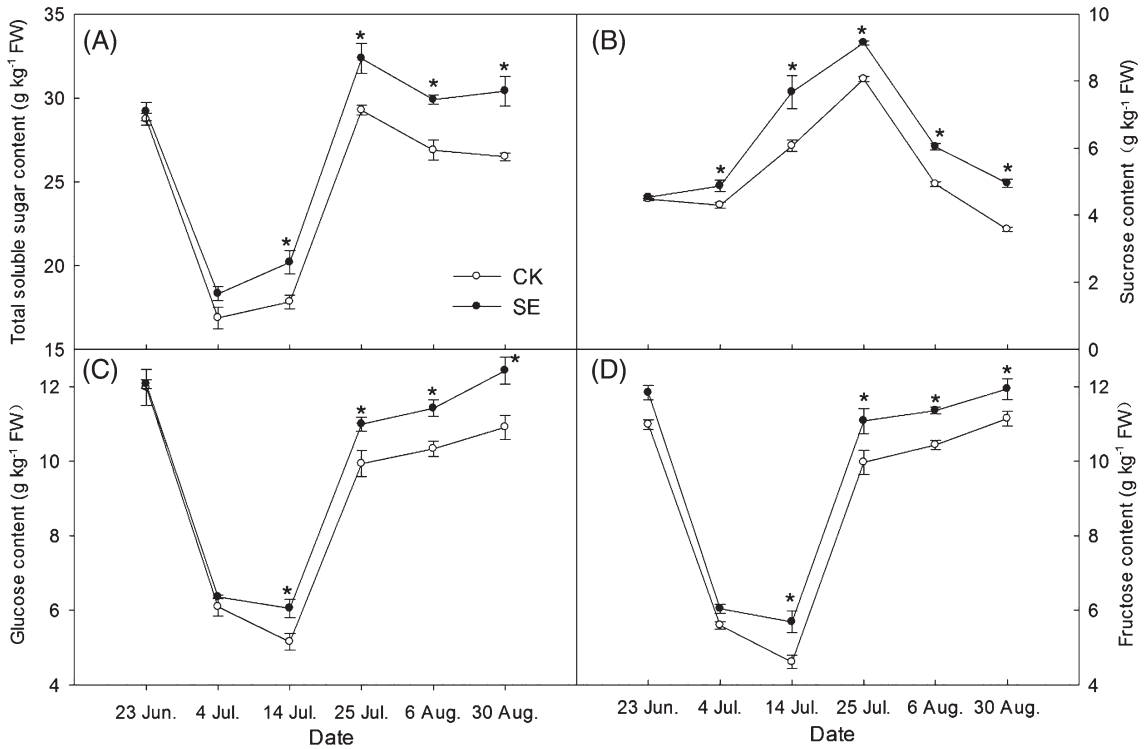

Figure 4. Developmental changes in sucrose, glucose, fructose and total soluble sugar content for control (CK) and Se fertilizer treatment (SE) in grape leaves during berry growth and development. Vertical bars represent standard error. Asterisk shows significant differences ($P < 0.05$) between CK and SE groups.

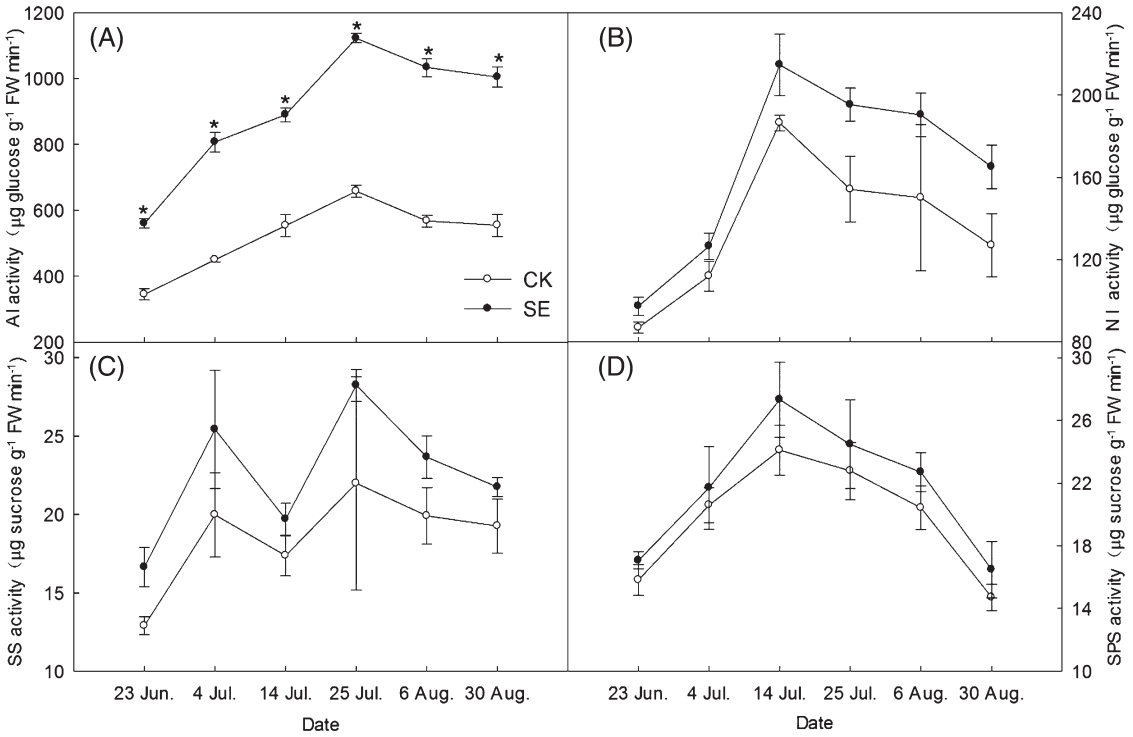

Figure 5. Developmental changes in acid invertase (AI), neutral invertase (NI), sucrose synthase (SS) and sucrose phosphate synthase (SPS) activities for control (CK) and Se fertilizer treatment (SE) in grape berries during berry growth and development. Vertical bars represent standard error. Asterisk shows significant differences (*$P < 0.05$) between CK and SE groups.

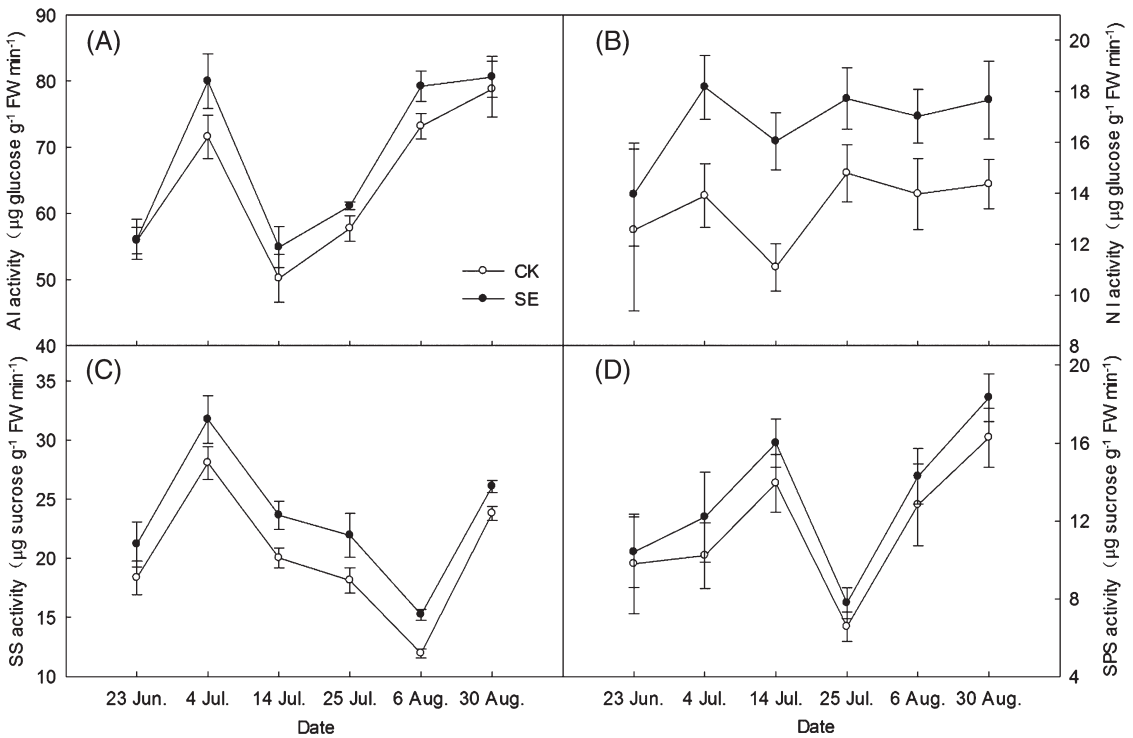

Figure 6. Developmental changes in acid invertase (AI), neutral invertase (NI), sucrose synthase (SS) and sucrose phosphate synthase (SPS) activities for control (CK) and Se fertilizer treatment (SE) in grape leaves during berry growth and development. Vertical bars represent standard error. Asterisk shows significant differences (*$P < 0.05$) between CK and SE groups.

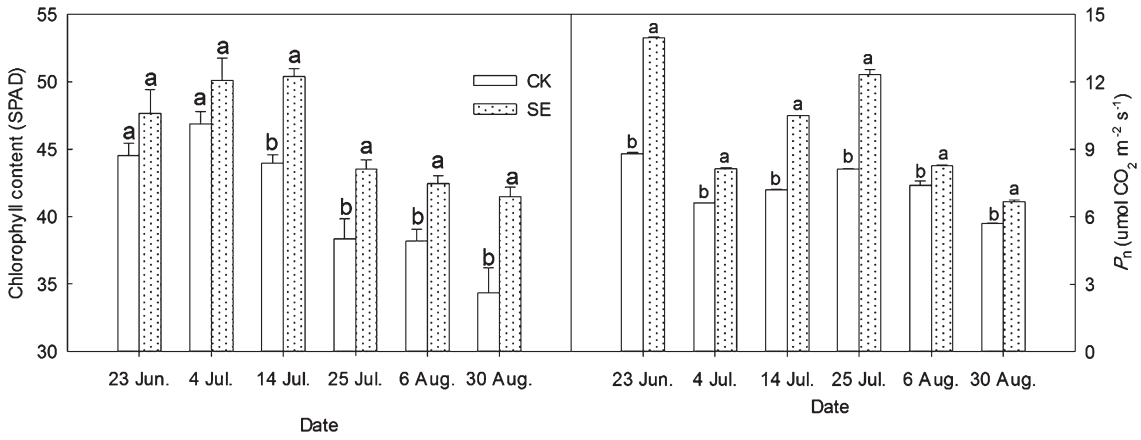

Figure 7. Changes in chlorophyll content and photosynthetic rate (P_n) in leaf for control (CK) and Se fertilizer treatment (SE) during berry growth and development. Different letters above columns show significant differences ($P < 0.05$) between CK and SE groups.

However, chlorophyll content in leaves later peaked for the SE group on 14 July, and on 4 July for the CK group (Fig. 7). Chlorophyll content was significant higher for the SE group compared to CK except on 23 June and 4 July ($P < 0.05$). Hence Se fertilizer helps to slow senescence of leaves, which sustain a high chlorophyll content for a longer period.

The patterns of change in photosynthetic rate (P_n) were similar in both SE and CK groups, and the pattern showed a volatility change during the whole growth period, ranging from 5.69 to 8.82 μmol CO_2 m^{-2} s^{-1} for CK and from 6.69 to 13.97 μmol CO_2 m^{-2} s^{-1} for the SE group (Fig. 7). Variance analysis showed that P_n in SE was significant higher than that in CK ($P < 0.05$).

Correlation analysis of different sugar components in berries and leaves

The total soluble sugar content in berries showed a significant correlation with sugar (sucrose, glucose and fructose) content in grape berries for both CK and SE groups – $r = 0.834^*$, 0.993^{**}, 0.997^{**}(CK) and $r = 0.854^*$, 0.992^{**}, 0.999^{**} (SE) – but it was not correlated with sugar (sucrose, glucose and fructose) content in leaves. There was significant correlation between glucose and fructose content in berries for both CK and SE groups, $r = 0.981^{**}$ (CK) and $r = 0.988^{**}$ (SE); also glucose and fructose content in leaves showed a significant correlation – $r = 0.987^{**}$ (CK) and $r = 0.998^{**}$ (SE) – in addition, fructose content in berries was correlated with sucrose content for both treatments. The total soluble sugar in leaves was significantly correlated with sugar (glucose and fructose) content in berries for both CK and SE groups, but there was no significant correlation with sucrose sugar (data not shown). Hence glucose and fructose constitute the predominant sugars in grape berries and leaves, and sucrose is imported into the grape berry from the leaves.

Table 2. Correlation analysis of different sugar components in grape berries and relative enzymes activities in berries and leaves in Se fertilizer-treated and control grapevines

	Fruit berries		Leaves	
Relationship	Control	Se fertilizer	Control	Se fertilizer
Sucrose–AI	0.595	0.739	0.715	0.608
Sucrose–NI	0.199	0.409	0.621	0.510
Sucrose–SS	0.534	0.343	−0.243	−0.308
Sucrose–SPS	−0.306	−0.249	0.470	0.432
Glucose–AI	0.916*	0.890*	0.215	0.235
Glucose–NI	0.751	0.868*	0.280	0.512
Glucose–SS	0.643	0.368	−0.371	−0.295
Glucose–SPS	0.270	0.322	0.354	0.475
Fructose–AI	0.836*	0.860*	0.326	0.314
Fructose–NI	0.643	0.786	0.304	0.488
Fructose–SS	0.567	0.318	−0.331	−0.322
Fructose–SPS	0.089	0.177	0.477	0.521

AI, acid invertase; NI, neutral invertase; SS, sucrose synthase; SPS, sucrose phosphate synthase.
Asterisk indicates that correlation is significant at the *0.05 level.

DISCUSSION

The accumulation of sugar is one of the main features of the ripening process in grape berries and is a major commercial consideration for the grape grower.[22] Commercially, the TSS/TA ratio is regarded as the most reliable measure of fruit flavour.[23] In addition, this study showed that TA content in fruit grown under Se fertilizer was lower than that in control fruit, and grape berries produced higher TSS under Se fertilizer, leading to a higher TSS/TA ratio than control (Fig. 1). A previous study showed that spraying Se fertilizer improved soluble sugar content in wheat;[24] the result was consistent with our study. Meanwhile, addition of Se fertilizer reduced the organic acid in grape berries, and was confirmed in kumquat fruit.[25] Therefore, Se fertilizer can improve berry flavor by increasing the TSS/TA ratio.

The grape berry is the major sink organ in the grapevine and requires carbohydrates to support its growth and development. Sugar metabolism plays an important role in berry growth and development.[26] This study showed that Se fertilizer increased sugar content in grape berries by promoting photosynthate accumulation, which was accompanied by changes in the activity of invertases. A study conducted by Swanson and Shishiny[2] showed that sucrose produced by leaf photosynthesis was imported via the phloem into the grape berry. After unloading of sucrose in the berry, invertases degraded sucrose and generated glucose and fructose at a suitable pH.[3] Glucose and fructose constitute the predominant sugars in grape berries. Although AI and NI activity for both treatments increased over the whole growth period, AI activity was higher than NI activity. A previous study had shown

that AI was most active where sucrose exited the transport path, rather than at sites of eventual storage.[20] Early in fruit development any sucrose that was synthesized appeared to be rapidly degraded, primarily by acid invertase, thus preventing sucrose accumulation.[27]

In tomato fruit, invertases have been reported to affect both total sugar content and sugar composition, particularly the hexose-to-sucrose ratio.[28] Similarly, in the present study, glucose content in berries and AI activity in berries showed a significantly positive correlation for both CK and SE groups, $r = 0.916^*$ (CK) and $r = 0.890^*$ (SE) (Table 2). Also, glucose content in berries and NI activity in berries showed a significant correlation under SE ($r = 0.868^*$). In addition, there was no significant correlation between sucrose content in berries and AI, NI, SS, SPS activity in grape berries and leaves for both treatments (Table 2); glucose content in berries was not correlated with SS, SPS activity in berries or leaves, or AI and NI in leaves (Table 2). Fructose content in berries and AI activity in berries was significantly positively correlated for both CK and SE, $r = 0.836^*$ (CK) and $r = 0.860^*$ (SE) (Table 2). However, it was not correlated with AI activity in leaves, or NI, SS, SPS in grape berries or leaves. There was no significant correlation between sugar (sucrose, glucose and fructose) content in leaves or AI, NI, SS, SPS activity in leaves for either SE or CK groups (data not shown). Consequently, AI can be regarded as the key restriction enzyme to the accumulation of sugar; increase of AI activity in berries in the SE group was an important reason for sugar increase in berries, while SS and SPS were not the key restriction enzymes in sugar accumulation. In addition, there was a significant difference between CK and SE for AI activity of berries ($P < 0.05$) (Fig. 4A). However, no linear correlations were observed between SS or SPS activity of berries and sugar (sucrose, glucose or fructose) content (Table 1). This finding was in agreement with another study showing that invertase was the main sucrose-hydrolysing enzyme in strawberry fruits and was substantially higher than SS or SPS activity.[29] In a recent study, Desnoues *et al.*[30] provided an overview of genetic control of sugar metabolism during peach fruit development using dynamic QTL for sugars and enzyme activities. Also, patterns of enzyme activities and gene expressions in sucrose metabolism in relation to sugar accumulation and composition in the aril of *Litchi* have been studied.[31] Thus, in order to study the interaction mechanism of sugar accumulation, related enzyme activities and Se content regarding gene transcripts of grape berries, vast field experiments are needed.

Studies have shown that Se fertilizer application can effectively increase Se content of crop edible parts.[32] Broadley *et al.* reported that by increasing Se fertilizer in wheat, Se content increased 10-fold in wheat grains compared with control; also, both soil application and foliar spray significantly improved Se content.[10,33] However, those studies mostly selected inorganic Se (Na_2SeO_3 and Na_2SeO_4) as the main form of Se fertilizer. With inorganic Se dual effects on plants, the excessive Se addition inhibited plant growth, resulting in reduced plant growth and a high mortality rate.[34] Thus inorganic Se fertilizer is not suitable for Se fertilizer application. This study concluded that spraying a 500× dilution of amino acid-chelated Se-enriched foliar fertilizer, which plants absorbed easily and with no harm to plants or humans, has contributed to increasing the Se content of grape berries.

Although Se fertilizer increased the Se content along with significantly increasing the sugar accumulation and affecting sugar metabolism, the process is complex, and there are no previous reports on the effects of Se fertilizer on sugar metabolism. A previous study[35] showed that Se fertilizer improves photosynthesis and protects photosystem II in grape, thus explaining the reason for Se content and sugar content increasing in the Se fertilizer-treated group. Many studies have reported effects of Se fertilizer on fruit quality, Se content and physiological characteristics in grape berry.[36,37] Some studies reported that mineral fertilizers also affect sugar metabolism and fruit quality.[38] Potassium accumulation contributes to tissue expansion growth and organic acid charge balance in the vacuole.[39] Many reports showed that potassium fertilization increases fruit TSS.[40,41] Nitrogen fertilizer application in apple trees influences fruit quality.[42] Therefore, further investigation of the application of organic and/or mineral fertilizers and enzymatic activity changes associated with grape berries produced under Se fertilizer may provide addition insights into mechanisms affecting sugar accumulation.

CONCLUSION

Foliar Se fertilizer not only can increase the Se content in fruit, but also could promote berry growth and sugar accumulation, improving internal berry quality. At maturity, the total soluble sugar content of grape berries in the Se fertilizer-treated group was 166.72 g kg^{-1}, which was significantly higher than the control 147.52 g kg^{-1}; glucose and fructose were the primary sugars in grape berries, with a trace amount of sucrose. AI played an important role in the process of sugar accumulation in grape berries; high AI activity in grape berries and high photosynthetic rate could explain the mechanism by which Se fertilizer affected sugar accumulation in grape berries, accordingly providing scientific guidance for foliar Se fertilizer application in high-quality Se-enriched grapes production.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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