Original article

Response of fruit quality of table grape (*Vitis vinifera* L.) to foliar selenium fertilizer under different cultivation microclimates

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Summary

Spraying selenium (Se) fertilizer is a new practice and an effective way for high quality Se-enriched fruit production. Cultivation microclimate affects fruit quality and alters physiological response of plants to fertilizers. This study designed control (CK) and Se fertilizer (SE) treatments with Hutai No.8 and Summer Black in greenhouse and open field respectively in 2016 and 2017. Results showed: Se content of grape berries was increased by 26.72% to 37.25% with Se fertilizer application among all treatments. In greenhouse, spraying Se fertilizer was conducive to improving fruit appearance traits, such as fruit shape index and fruit color. Spraying Se fertilizer increased the content of soluble sugars, soluble proteins, TSS, Vc, RES, OPC, and decreased TA content of grape berries in both greenhouse and open field. To a certain extent, either planting in greenhouse or spraying Se fertilizer could improve fruit health care values (Vc, RES, OPC and Se content), nutritional values (soluble sugars, soluble proteins) and flavor values (TSS and TA) for both two varieties. Comprehensive evaluation indicated that fruit quality of Hutai No.8 was better than that of Summer Black, also among different treatments HG-SE > HO-SE > HG-CK > HO-CK for Hutai No.8, and SG-SE > SO-SE > SG-CK > SO-CK for Summer Black. Therefore, spraying Se fertilizer and planting in greenhouse were effective measures to improve fruit quality of grape production in the future.

Keywords

China, *Vitis*, fertilization, physical properties, chemical properties, environment

Abbreviations

CK, control treatment; SE, selenium fertilizer treatment; HO, Hutai No.8 in open field; HG, Hutai No.8 in greenhouse; SO, Summer Black in open field; SG, Summer Black in greenhouse; Se, selenium; Vc, vitamin C; RES, resveratrol; OPC, proanthocyanidin; TSS, total soluble solids; TA, titratable acidity.

Introduction

Table grape is a fruit well suited for consumption as fresh-cut product because of their attractiveness, large amount of nutrition content and pharmacological properties (Yadav et al., 2009). It contains soluble sugars, soluble proteins, vitamins, organic acids, resveratrol, proanthocyanidin and so

Significance of this study

What is already known on this subject?

- Selenium fertilizer application is an effective way for high quality Se-enriched fruit production, and cultivation microclimate affects fruit quality and alters physiological response of plants to fertilizers.

What are the new findings?

- Foliar Se fertilizer increased Se content of grape berries, and improved fruit shape index and fruit color in greenhouse; fruit quality of grape berries in greenhouse was superior to that in open field; foliar Se fertilizer in open field could achieve the same fruit quality effect on control of greenhouse.

What is the expected impact on horticulture?

- Spraying Se fertilizer and planting in greenhouse should be considered by viticulture in order to improve fruit quality in the future.

on. However, the grape quality depends on many factors including vineyard management, cultivar and harvest time etc. (Rizzuti et al., 2015). In addition, grape is one of the major sources of phenolic compounds among multitudinous fruits (Xia et al., 2010). The proanthocyanidin and resveratrol possess many biological activities, and have a very good medical and health care value in the aspect of preventing cardiovascular disease and cancer mortality (Kiskova et al., 2014; Li et al., 2014; Wallerath et al., 2002), anti-aging (Rice-Evans, 2001) activities, anti-inflammatory (Ghanim et al., 2010; Landolfi et al., 1984; Moroney et al., 1988), antioxidant (Bors and Saran, 1987; Chang et al., 1977), inhibition of platelet aggregation, antimicrobial (Capasso et al., 1995; Wauwe and Goossens, 1983).

Selenium (Se) is an essential trace element in human nutrition that has been demonstrated to be effective in reducing the risk of cancer, scavenging free radicals, resisting to membrane lipid peroxidation, enhancing the immune function in human (Keskinen et al., 2009). Se deficiency is a serious nutritional and health problem, while approximately two-thirds of the cultivated soil is Se-deficient in China (Zhang et al., 2014). Spraying Se fertilizer is a new practice for high quality production and an effective way of developing the function of Se-enriched fruit (Vashistha et al., 2010). Research had revealed that Se fertilization not only increased Se content, but did also not affect the yield in durum wheat (Grant et al., 2007). Besides, another study pointed out Se

fertilization was beneficial in increasing lentil seed yield (Ekanayake et al., 2015). Meanwhile, Se fertilizer application increased total proteins and amino acids, Vc, soluble sugar concentrations in potatoes, onions and green tea leaves (Hu et al., 2003; Kopsel and Randle, 1997; Munshi et al., 1990). Moreover, various studies all indicated that Se fertilizer can effectively increase Se content of crop edible parts (Broadley et al., 2010; Ekanayake et al., 2015) and reduce accumulation of heavy metals (Fargašová et al., 2006). But most of those studies selected inorganic Se (Na_2SeO_3 and/or Na_2SeO_4) as the main forms of Se fertilizer, and the majority focused on application method (Grant et al., 2007), fertilization rate on the crop quality and Se concentration (Curtin et al., 2008; Tremblay et al., 2014). However, in many Se-enriched plants, most Se is in its major organic form. Given that this form is more available to humans and more efficient in increasing Se content than inorganic forms. Foliar spray was found to be a safe and cost-effective means to increase the Se content of rice (Hu et al., 2002). Thus, our study adopts the organic amino acid-chelated Se-enriched foliar fertilizer as a source of Se fertilizer. In addition, study of spraying Se fertilizer on fruit quality of different cultivation microclimates had not been reported in detail. Environmental factors (air temperature, relative humidity, light intensity) had a combined effect on grapevine physiology, yield and quality (Keller et al., 2008; Logan et al., 2010; Zsófi et al., 2011). Meanwhile, cultivation microclimates profoundly affect fruit quality and can alter physiological response of plants to fertilizers, which had not been reported in detail accompanied with Se fertilizer. Therefore, the influences of Se fertilizer under different microclimates on grape quality had attracted considerable interest.

Above all, the objective of this study was to explore the fruit quality changes caused by exogenous organic Se fertilizer under different cultivation microclimates, and to evaluate fruit quality of grape berries comprehensively, to choose the optimum planting pattern for high quality Se-enriched grape, which could provide scientific guidance of grape production and cultivation.

Materials and methods

Experiment design

The experiment was carried out during two consecutive vintages in 2016 and 2017 at forestry and fruit industry demonstration orchard of grapes, a traditional fruit tree culture region, located at Xianyang ($34^{\circ}21'N$; $108^{\circ}38'E$), Shaanxi province of China. The soil texture was Cumulic Cinnamon soil (27.4% sand, 52.9% silt, and 19.7% clay, on average) with a pH value of 7.8; 0–30 cm soil bulk density was 1.29 g cm^{-3} ; soil water holding capacity was 240 g kg^{-1} (mass basis); organic matter content was 9.80 g kg^{-1} ; total N content was 0.56 g kg^{-1} ; total P content was 0.72 g kg^{-1} ; available N (1 mol L^{-1} NaOH hydrolysis) was 9.63 mg kg^{-1} ; available P (0.5 mol L^{-1} NaHCO_3) was 26.93 mg kg^{-1} ; available K (1 mol L^{-1} neutral NH_4OAc) was $162.37 \text{ mg kg}^{-1}$; selenium content (flame atomic absorption method) was 68 ug kg^{-1} . The air temperature (T) and air relative humidity (RH) were recorded every 2 hours from 00:00 automatically by HOB0 (HOB0 UX100-011, Onset Computer Corporation, USA), which was located in the middle of the plot area. The solar radiation was recorded every 2 hours from 08:00 to 18:00 automatically by CR10X-2M data collector and LI200X silicon crystals radiation sensor (Campbell Scientific Co., Ltd., USA), and average values were shown in Figure 1.

FIGURE 1. Variation of air temperature (T), air relative humidity (RH) and solar radiation during grape growth stage in greenhouse and open field.

Experiment was performed on Hutai No.8 and Summer Black, which were grown using stem cutting (no rootstock) in greenhouse and open field on 6 December 2011, then transplanting to greenhouse and open field in the spring of 2013. In 2016 and 2017, different processing were conducted, including control (CK, pure water) and Se fertilizer treatments (SE, amino acid-chelated Se-enriched foliar fertilizer, contains Se, Ti and a variety of chelating trace elements, $\text{Cu}+\text{Fe}+\text{Mn}+\text{Zn}+\text{B} \geq 100 \text{ g L}^{-1}$, organic $\text{Se} \geq 60 \text{ g L}^{-1}$, and it was provided by Shaanxi Yangling Macao Bond Biological Science Co., Ltd.). Se fertilizer, i.e., aforesaid mixture which was diluted 500 times with pure water, was applied as a foliar spray when the berries were at 20, 34 and 46 days after fruit set during evening hours (17:00 to 18:00) between May and June in 2016 and 2017 respectively, totally three times. Similarly, pure water was applied on control vines. All the vines were oriented in the north-south direction, and the planting density was 2.5 m between rows and 0.8 m between vines in each plot, which composed of 50 vines of the said varieties. The experimental design was randomized complete block design with three replications of each treatment, totally 12 plots in greenhouse and 12 plots in open field respectively. All plantation was managed following the usual local procedures, organic fertilizer was applied 180 g per tree, K fertil-

TABLE 1. The different treatments in 2016 and 2017.

Cultivation variety	Cultivation microclimate	Control (CK)	Selenium fertilizer (SE)
Hutai No.8	Open field	HO-CK	HO-SE
	Greenhouse	HG-CK	HG-SE
Summer Black	Open field	SO-CK	SO-SE
	Greenhouse	SG-CK	SG-SE

Notes: HO, Hutai No.8 in open field; HG, Hutai No.8 in greenhouse; SO, Summer Black in open field; SG, Summer Black in greenhouse; CK, control treatment; SE, Se fertilizer treatment.

izer and urea were applied at the dose 40 g and 20 g per tree respectively. The plots were irrigated mildly after each fertilization. All treatments were shown in Table 1.

Measurement methods of grape samples

1. Sampling of grape berries. Samples of Hutai No.8 in greenhouse and open field were collected at maturity on 5, 18 August 2016 and 9, 25 August 2017 respectively, samples of Summer Black in greenhouse and open field were collected at maturity on 15, 30 July 2016 and 22 July, 9 August 2017 respectively. Twenty clusters of maturity grape berries that were normal growth and not injured, were gathered randomly in different positions and directions of each plot to avoid fruit position effect, and 20 fruits were selected randomly in each cluster. Portion of the samplings were used in determination of grape physical properties, and the remaining were rinsed with distilled water three times and dried out surface water at normal temperature, then pressed into homogenate by a juicer and put in glass bottle at 4°C refrigerator for the analysis of grape chemical composition.

2. Grape physical properties. Vertical and transverse diameter of ten berries in each repeating group were measured with vernier caliper, then the ratio of vertical and transverse diameter expressed as fruit shape index (Sun et al., 2012). Fruit color (L^* , a^* , b^*) was measured by Konika-Minolta (Chroma Meter CR-400, Minolta Corporation, Ltd., Osaka, Japan) portable handheld colorimeter according to the international common Hunter scale, five fruits from each repeating group were selected, measurements were made at equatorial plane of each fruit (Miceli et al., 2014), then were calculated the comprehensive indexes of chromaticity, including chromaticity angle (H), color saturation (C) and color ratio (h).

3. Grape chemical composition. All reagents used were of analytically pure reagents, and deionized water was used for all chemical preparations and dilutions. Total soluble solids (TSS, expressed in °Brix) were determined by an ATAGO PR-101 digital refractometer (ATC-1 Atago, Japan) and titratable acidity (TA) by titration with 0.1 N NaOH until the pH value of the solution reached 8.2. Total soluble sugars were measured by phenol-sulfuric acid method (Kochert, 1978). Molybdenum blue colorimetric method was used to determine vitamin C (Vc) content (Gao, 2006). The content of soluble proteins were measured as follows: approximately 2 g of homogenate sample was put into a 10-mL centrifuge tube, and 6 mL distilled water was added. The centrifuge tube maintained standing for 30 min, then centrifuged and abandoned the sediment. The supernatant was retained for analysis of soluble proteins using Coomassie Brilliant Blue G-250 method (Bradford, 1976). Proanthocyanidin (OPC) content was measured approximate fairly the original Porter (Porter et al., 1986) method: approximately 2 g of homogenate sample was put into a centrifuge tube and adding with absolute ethyl

alcohol to 10 mL, then standing for 30 min. The supernatant was analysed by molybdate catalytic colorimetry method (Porter et al., 1986) with a spectrophotometer (Bibby Jenway 6305, Britain) at 525 nm. The content of resveratrol (RES) was measured with potassium permanganate fading spectrophotometry according to Jiang et al. (Jiang and Liu, 2009): 3 g of homogenate sample was put into a centrifuge tube, and adding with absolute ethyl alcohol to 10 mL, then ultrasonic extraction for 30 min. Later, centrifuged at $5,000 \times g$ for 15 min, the supernatant was analysed with a spectrophotometer (Bibby Jenway 6305, Britain) at 525 nm.

The total Se concentration was determined using a method similar to Premarathna and Toaldo (Premarathna et al., 2012; Toaldo et al., 2013). Homogenate sample was digested using a closed vessel microwave procedure (CEM, USA) with a three-stage time program: 5 min at 300 W, 10 min at 500 W and 25 min at 800 W. Approximately 2.5 g of fine homogenate sample was transferred into a Teflon digestion vessel and 10 mL of concentrated HNO_3 acid and 2 mL H_2O_2 were added. After the solution of microwave digestion was about 1 mL, the vessel was allowed to cool at room temperature and then diluted to 10 mL with ultrapure deionized water (Milli-Q, Millipore, Bedford, MA). Digest solution was analysed for total Se concentrations by inductively coupled plasma-mass spectrometry (ICP-MS) (820-MS, Varian, USA).

Statistical analysis

The data were analyzed with version 20.0 of SPSS (SPSS Inc., Chicago, USA). Statistical significant differences ($p < 0.05$) from different treatments were revealed after one-way analysis of variance (ANOVA), and multiple comparison followed by Duncan test. Graphs were plotted using Sigma-Plot 12.5 (Systat Software, San Jose, CA).

The statistical analysis data of each quality index comes from the mean of two consecutive vintages in 2016 and 2017. Assessed the subordinate function values of each index (selenium content, TSS, soluble sugar, soluble protein, Vc, RES and OPC) by subordinate function values analysis, the formula was:

$$U(x_i) = (X_i - X_{\min}) / (X_{\max} - X_{\min})$$

Type: $U(x_i)$ is the subordinate function value, x_i is measurement of an index, $X_{\max}$ and $X_{\min}$ is maximum and minimum values in an index of all the treatments.

The anti-subordinate function values analysis transformation was used if an index (TA) that comprehensive evaluation results showed a negative correlation, formula was:

$$U(x_i) = 1 - (X_i - X_{\min}) / (X_{\max} - X_{\min})$$

The synthesis score of all indexes was used to evaluate the grape quality of different treatments.

FIGURE 2. Se content in grape berries of different treatments. Note: CK, control treatment; SE, Se fertilizer treatment; HO, Hutai No.8 in open field; HG, Hutai No.8 in greenhouse; SO, Summer Black in open field; SG, Summer Black in greenhouse. The different letters above pillars show significant differences ($p < 0.05$) between CK and SE treatments.

Results

Selenium content in grape berries

During two consecutive vintages, spraying Se fertilizer significantly increased Se content of grape berries in both open field and greenhouse ($p < 0.05$), and Se content of grape berries in greenhouse was significant higher than that in open field for both Hutai No.8 and Summer Black under SE treatments ($p < 0.05$) (Figure 2). Hutai No.8 had higher Se content in grape berries when compared to Summer Black, and Se content of grape berries was increased on average 27.21% and 26.72% for open field and greenhouse respectively in

2016 and 2017. Similar to Summer Black, the average raised rates were 32.57% and 37.25%. And the mean values of Se content in grape berries under SE treatments reached 24.76 $\mu\text{g kg}^{-1}$ to 51.87 $\mu\text{g kg}^{-1}$ during the two consecutive vintages, which meet the standard of the Se-enriched fruits.

Variation of grape appearance traits under different treatments

Grape berry appearance traits were measured as changes in chromaticity angle (H), color saturation (C) and color ratio (h) and fruit shape index for both control and Se fertilizer treatments of Hutai No.8 and Summer Black in greenhouse

FIGURE 3. The appearance traits in H (chromaticity angle), C (saturation), h (color ratio), and fruit shape index for control (CK) and Se fertilizer treatment (SE) of Hutai No.8 and Summer Black in greenhouse and open field. Note: HO, Hutai No.8 in open field; HG, Hutai No.8 in greenhouse; SO, Summer Black in open field; SG, Summer Black in greenhouse. The different letters above pillars show significant differences ($p < 0.05$) between CK and SE treatments.

FIGURE 4. The flavor quality in TSS (total soluble solids) and TA (titratable acidity) for control (CK) and Se fertilizer treatment (SE) of Hutai No.8 and Summer Black in greenhouse and open field. Note: HO, Hutai No.8 in open field; HG, Hutai No.8 in greenhouse; SO, Summer Black in open field; SG, Summer Black in greenhouse. The different letters above pillars show significant differences ($p < 0.05$) among all treatments.

FIGURE 5. The nutritional quality in soluble sugars and soluble proteins for control (CK) and Se fertilizer treatment (SE) of Hutai No.8 and Summer Black in greenhouse and open field. Note: HO, Hutai No.8 in open field; HG, Hutai No.8 in greenhouse; SO, Summer Black in open field; SG, Summer Black in greenhouse. The different letters above pillars show significant differences ($p < 0.05$) among all treatments.

and open field (Figure 3). From the aspect of H (the smaller H, the deeper redness, $H=0$ on behalf of mulberry), there was no significant difference between control and Se fertilizer treatments for either Hutai No.8 or Summer Black in open field (HO and SO) ($p < 0.05$), but SE significantly decreased the H for Hutai No.8 and Summer Black in greenhouse (HG and SG) ($p < 0.05$) (Figure 3A). There was no significant difference in C (the bigger C, fruit color is more bright) between CK and SE except Hutai No.8 in open field (HO) ($p < 0.05$) (Figure 3B). From the aspect of h (the bigger h, the darker redness), Se fertilizer significantly increased h value of Hutai No.8 in both greenhouse and open field ($p < 0.05$); however, there was no significant difference between CK and SE for Summer Black in either greenhouse or open field ($p < 0.05$) (Figure 3C). Integrated H, C, h three indicators, fruit color in open field was more red and more brightness than that in greenhouse. Se fertilizer significantly increased the redness of Hutai No.8 in greenhouse (HG) and the brightness of Hutai No.8 in open field (HO) ($p < 0.05$). The fruit shape index under Se fertilizer was significant greater than that of the control for HG and SG ($p < 0.05$), but there was no significant difference between CK and SE for HO ($p < 0.05$), and the fruit shape index under SE treatment was significant lower compared with CK for SO ($p < 0.05$) (Figure 3D). In greenhouse, Se fertilizer decreased H and increased fruit shape index for

both Hutai No.8 and Summer Black, while there was no significant effect on C of grape berries.

Flavor values: TSS and TA

Spraying Se fertilizer significantly increased total soluble solid (TSS) content, meanwhile decreased the titratable acidity (TA) content for both Hutai No.8 and Summer Black during two consecutive vintages (Figure 4). Either CK or SE treatments, the TSS content of grape berries had significant difference between greenhouse and open field ($p < 0.05$), also TSS content in greenhouse was significantly higher than that in open field except for the Summer Black under CK treatment in 2017. Similarly, TA content of grape berries in greenhouse was higher than that in open field for both two grape varieties either CK or SE treatments during two consecutive vintages.

Nutritional values: soluble sugar and soluble protein

Foliar Se fertilizer significantly increased the soluble sugar content of grape berries for both Hutai No.8 and Summer Black in greenhouse and open field ($p < 0.05$) during two consecutive vintages in 2016 and 2017 (Figure 5). Except for the treatments of Summer Black variety in 2017, the soluble sugar content of grape berries had significant difference between greenhouse and open field for both two grape varieties

FIGURE 6. The health care quality in Vc (vitamin C), RES (resveratrol) and OPC (proanthocyanidin) for control (CK) and Se fertilizer treatment (SE) of Hutai No.8 and Summer Black in greenhouse and open field. Note: HO, Hutai No.8 in open field; HG, Hutai No.8 in greenhouse; SO, Summer Black in open field; SG, Summer Black in greenhouse. The different letters above pillars show significant differences ($p < 0.05$) among all treatments.

ies ($p < 0.05$), and they were higher in greenhouse than that in open field either CK or SE treatments (Figure 5). Similar to soluble sugar content, spraying Se fertilizer significantly increased protein content of grape berries in greenhouse and open field for both Hutai No.8 and Summer Black ($p < 0.05$) (Figure 5) and the protein content of greenhouse was higher than that in open field for two grape varieties on either CK or SE treatments during two consecutive vintages.

The health care values: Vc, RES, OPC

In 2016 and 2017, foliar Se fertilizer application significantly increased Vc content of grape berries for Hutai No.8 and Summer Black in both greenhouse and open field ($p < 0.05$) (Figure 6A). Vc content of Hutai No.8 was higher than that of Summer Black in both greenhouse and open field ($p < 0.05$); in addition, Vc content of grape berries in greenhouse was higher than that in open field for either Hutai No.8 or Summer Black ($p < 0.05$).

Either Hutai No.8 or Summer Black, the RES content of grape berries had no significant difference between greenhouse and open field under CK treatments in 2016 ($p > 0.05$) (Figure 6B). However, foliar Se fertilizer application significantly increased RES content of grape berries in open field ($p < 0.05$), also the RES content of HO-SE (SO-SE) was higher than that of HG-CK (SG-CK) in 2016. Similarly, foliar Se fertilizer application significantly increased RES content of grape berries in greenhouse for both two grape varieties in 2016

($p < 0.05$). In 2017, foliar selenium fertilizer application significantly increased RES content of grape berries for all treatments except for Summer Black in greenhouse.

During two consecutive vintages, OPC content of grape berries significantly increased under SE treatments compared to CK in both greenhouse and open field for either Hutai No.8 or Summer Black ($p < 0.05$) (Figure 6C). Meanwhile, the OPC content of Hutai No.8 was significantly higher than that of Summer Black ($p < 0.05$). As a result, from the aspect of variety, Hutai No.8 can be called a high-OPC and high-Vc grape variety, it was a good choice to produce table grapes that rich in health care values.

Comprehensive evaluation of fruit quality

The descriptive statistics of the different variables during two consecutive vintages were presented in Table 2, and the subordinate function values of each quality index were calculated in Table 3. Using the total subordinate function values of every index (synthesis score) to comprehensively evaluate the grape quality of each treatment, with higher score as indicative of better comprehensive fruit quality.

According to the comprehensive evaluation results (Table 3), the highest synthesis score of fruit quality was HG-SE, followed by HO-SE, while the lowest score was SO-CK, followed by SG-CK; in addition, the synthesis score of Hutai No.8 under CK treatments were higher than those of Summer Black in either greenhouse or open field. Similarly, the

TABLE 2. Descriptive statistics of the different variables during two consecutive vintages.

Variable	Min.	Max.	N	Mean	SD
Soluble sugar (g kg ⁻¹)	111.34	148.05	48	130.06	10.15
Soluble protein (g kg ⁻¹)	1.00	2.86	48	1.80	0.51
Vc (g kg ⁻¹)	0.08	0.23	48	0.15	0.04
TSS (%)	17.50	20.30	48	18.95	0.75
TA (%)	0.33	0.61	48	0.47	0.08
Se (ug kg ⁻¹)	17.27	55.79	48	33.31	11.51
RES (mg kg ⁻¹)	0.21	0.28	48	0.24	0.02
OPC (g kg ⁻¹)	1.06	9.98	48	5.40	3.10

Notes: Vc, vitamin C; TSS, total soluble solid; TA, titratable acidity; Se, selenium content; RES, resveratrol; OPC, proanthocyanidin. The statistical data come from two consecutive vintages in 2016 and 2017.

Min., minimum value of statistical data; Max., maximum value of statistical data; N, total number of sample statistic; Mean, average value of statistical data; SD, standard deviation of statistical data.

TABLE 3. Subordinate function values of fruit quality under different treatments.

Treatments	Fruit quality											Synthesis score
	Nutritional values			Flavor values			Health care values					
	Soluble sugar	Soluble protein	Score	TSS	TA	Score	Vc	Se	RES	OPC	Score	
HO-CK	0.00	0.23	0.23	0.00	0.79	0.79	0.53	0.51	0.00	0.77	1.81	2.83
HO-SE	0.42	0.79	1.21	0.23	1.00	1.23	0.72	0.81	0.29	0.94	2.75	5.20
HG-CK	0.63	0.48	1.11	0.43	0.30	0.72	0.74	0.67	0.07	0.68	2.15	3.99
HG-SE	1.00	1.00	2.00	0.77	0.42	1.19	1.00	1.00	0.29	1.00	3.29	6.47
SO-CK	0.27	0.00	0.27	0.51	0.23	0.74	0.00	0.00	0.57	0.00	0.57	1.59
SO-SE	0.76	0.35	1.11	0.76	0.49	1.25	0.17	0.18	1.00	0.10	1.45	3.81
SG-CK	0.20	0.21	0.41	0.47	0.00	0.47	0.24	0.06	0.29	0.17	0.76	1.64
SG-SE	0.62	0.43	1.05	1.00	0.27	1.27	0.38	0.29	0.68	0.27	1.62	3.94

Notes: HO, Hutai No.8 in open field; HG, Hutai No.8 in greenhouse; SO, Summer Black in open field; SG, Summer Black in greenhouse; CK, control treatment; SE, Se fertilizer treatment. Vc, vitamin C; TSS, total soluble solid; TA, titratable acidity; Se, selenium content; RES, resveratrol; OPC, proanthocyanidin. The statistical data come from the mean of two consecutive vintages in 2016 and 2017.

synthesis score of Hutai No.8 under SE treatments were still higher than those of Summer Black. Therefore, fruit quality of Hutai No.8 was superior to that of Summer Black.

The synthesis score of fruit quality under SE treatments were higher than those under CK treatments in both greenhouse and open field for two grape varieties. Either Hutai No.8 or Summer Black, synthesis score of fruit quality in open field was lower than that in greenhouse under CK treatments, while the synthesis score of fruit quality that foliar Se fertilizer in open field was higher than CK treatments in greenhouse (Table 3). Therefore, spraying Se fertilizer in open field can achieve the same effect on fruit quality compared to that in greenhouse under CK treatments. Additionally, the synthesis score of fruit quality in greenhouse of SE treatments was higher than that in open field of SE treatments.

From the perspective of fruit health care values (Vc, Se, RES and OPC) (Table 3), fruit quality of Hutai No.8 was superior to that of Summer Black; besides, fruit quality of SE treatments were superior to those of CK for both varieties; fruit quality in greenhouse were better than those in open field either CK or SE treatments. Therefore, planting in greenhouse and spraying Se fertilizer were effective measures to improve fruit health care benefits.

From the perspective of fruit nutritional values (soluble sugar and soluble protein) (Table 3), Se fertilizer could improve the content of fruit nutrient for both Hutai No.8 and Summer Black in greenhouse and open field. The nutritional composition content of Hutai No.8 was the highest in greenhouse under SE treatment (HG-SE), while Summer Black had the highest nutritional composition content in open field under SE treatment (SO-SE). Therefore, spraying Se fertilizer was an effective measure to improve fruit nutritional values.

From the perspective of fruit flavor (TSS and TA) (Table 3), fruit flavor value in open field was superior to that in greenhouse under CK treatments for both two grape varieties. In addition, spraying Se fertilizer in greenhouse could improve the fruit flavor quality for both Hutai No.8 and Summer Black so as to achieve the effect in open field under CK treatments.

Discussion

Enhanced Se content of grape berries by foliar Se fertilizer application

Many studies had shown that Se fertilizer application can effectively increase Se content of crop edible part, such as Broadley et al. (2010) by increasing Se fertilizer on wheat; Se content increased by 10-folds in wheat grains compared with the control. Both soil application and foliar spray significantly increased Se content (Broadley et al., 2010; Wang et al., 2013). However, those studies mostly selected inorganic Se as the main forms of Se fertilizer. Excessive inorganic Se addition inhibited plant growth, and even might lead to plant death (Pezzarossa et al., 2009). Therefore, this study concluded that spraying 500 times dilution of amino acid-chelated Se-enriched foliar fertilizer, at which rate plants absorbed easily and there was no harm for plants or humans, contributed to increasing the Se content of grape berries. In addition, it could meet the requirements of Se-enriched fruit production. The results of this study was consistent with the author's previous research (Zhu et al., 2017). However, crop variety affects Se efficiency in plants (Rengel et al., 1999). Similarly, this study found that Hutai No.8 was easier to absorb and accumulate Se than Summer Black, which indicated that variety selection was a key factor for Se-enriched grape production.

Effect of Se fertilizer and cultivation microclimates on fruit quality

Fruit quality is a comprehensive trait (Dai et al., 2010). But researches cannot contain all quality indices in the process of evaluation. This study took fruit color and fruit shape index as grape appearance traits, took soluble sugar, soluble protein, Vc, TSS, TA, Se content as well as the important secondary metabolites OPC and RES as fruit quality index. Some studies had reported that minerals fertilizer affect fruit quality (Marzouk et al., 2011), such as the potassium fertilizer contributes to tissue expansion growth in the vacuole (Lobit et al., 2003). This study showed that spraying Se fertilizer increased the fruit shape index in greenhouse. On the contrary, in open field it had no significant effect for Hutai No.8, despite the reduced fruit shape index for Summer Black (Figure 2D), which may be caused by the differences in microclimate factors and varieties. Similarly, for fruit quality, the present study showed that Se fertilizer application improved amino acids, proteins, soluble sugars, Vc in potatoes, onions and green tea leaves (Hu et al., 2003; Kopsel and Randle, 1997; Munshi et al., 1990). Dhillon (2009) did similar research on wheat, which improved the nutrient content of crop such as proteins, soluble solids, soluble sugars (Dhillon and Dhillon, 2009). This was in accordance with our study that Se fertilizer improved the fruit quality, such as soluble proteins, TSS, soluble sugars etc. (Figures 4 and 5). In addition, there were almost no reports about the effects of spraying Se fertilizer on the content of RES and OPC in grapes, so this study was more necessary.

Studies had shown that light is the main factor affecting fruit coloring (Tao et al., 2003), and previous research mostly involved in improving the fruit appearance properties by strengthening the nutrition and water management, regulating the environmental conditions, and changing the time of fruit harvest (Giménez et al., 2015; Roupheal et al., 2010). With the higher color ratio, the deeper redness (Miceli et al., 2014), this study concluded that the fruit redness of open field was deeper than that of greenhouse, which is mainly caused by the relatively sufficient light resources and solar radiation (Table 1) in open field. Therefore, when grapes were planted in a greenhouse, grape growers should take measures to enhance the light, such as picking leaves, artificial light, etc., for ensuring good fruit appearance traits. In this study, spraying Se fertilizer in greenhouse also proved to be an effective measure to improving fruit appearance traits.

Commercially, the TSS/TA ratio is regarded as the most reliable measure of fruit flavor in horticulture, the higher TSS/TA ratio, the better flavor value (Montero et al., 1996; Saradhuldhath and Paull, 2007). In this study, the scores of fruit flavor values of open field were better than that of greenhouse under all CK treatments. This is mainly due to the relatively sufficient light resources of cultivation in open field, which was conducive to the accumulation for photosynthetic products of TSS. Similarly, previous studies had shown that antioxidant phenolic in grape berries had large differences on the basis of different varieties, cultivation conditions, climate conditions, and so on (Locatelli et al., 2016; Ojeda et al., 2002). The result was consistent with our study, the health care values in greenhouse were significantly higher than those in open field, and Hutai No.8 had higher health care values than Summer Black (Figure 2 and 5, Table 3). Though various studies demonstrated that Se fertilizer application could increase the Se content and improve fruit quality (Broadley et al., 2010; Ekanayake et al., 2015). However, in order to study the interaction mechanism of Se fertilizer and microclimate on each quality index of grape berries, vast

field experiments were needed, so as to achieve Se-enriched fruits production accompanied with the high fruit quality.

Comprehensive evaluation in fruit quality

This study adopted subordinate function of fuzzy mathematics method to evaluate fruit quality of eight different treatments from three aspects. It could reflect a comprehensive level of multiple components in comprehensive characters, and this method had more persuasion relative to single contrast of each fruit quality index. The optimal treatment with highest synthesis score was selected, which at last an excellent cultivation management pattern of Se-enriched was acquired. Fruit quality of SE treatments were better than those of CK treatments, which was consistent with the previous research that Se fertilizer improved fruit quality (Premarathna et al., 2012; Zhu et al., 2017). Fruit quality in greenhouse was better than that in open field. This was opposite to the traditional idea that vegetables and fruits in greenhouse were regarded as the bad taste, which might be due to this study selected Se content as well as the secondary metabolites of RES and OPC that participated into the evaluation system.

Conclusion

In greenhouse, spraying Se fertilizer was an effective measure to improve fruit quality of both Hutai No.8 and Summer Black due to improving fruit appearance traits, increasing Se content. The fruit health care values of Hutai No.8 were superior to that of Summer Black, and that in greenhouse were better than that in open field. Comprehensive quality effect were HG-SE > HO-SE > HG-CK > HO-CK for Hutai No.8, and SG-SE > SO-SE > SG-CK > SO-CK for Summer Black. Therefore, spraying Se fertilizer and planting in a greenhouse were effective measures to improve fruit quality. Accordingly, providing scientific guidance for foliar Se fertilizer application in high quality Se-enriched grape production.

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