Effects of calcium on the formation of berry russet and phenolic compounds in ‘Shine Muscat’ grape

Yan Huang 1, Pei Wang 2, Xiao Wang 2, Jin Wang 2 *, Fang Liu 3 *

1Chengdu academy of agricultural and forestry sciences, Chengdu, Sichuan, 611130, China
2Institute of Pomology and Olericulture, Sichuan Agricultural University, Chengdu, Sichuan, 611130, China
3Leshan Normal University, Leshan, Sichuan, 614000, China
*Corresponding author’s e-mail: 14224@sicau.edu.cn; 308016@jsnu.edu.cn

Abstract. Four year old ‘Shine Muscat’ grape was used as the test material to spray amino acid calcium and water-soluble calcium solution on grape leaves and ears during fruit expansion and turn-color stages. A randomized block test was conducted to study the effects of exogenous calcium fertilizer on the formation of fruit russet and phenolic compounds in ‘Shine Muscat’ grape. The test was used to set the concentration of amino acid calcium solution to 1000 times, and to set the concentration of calcium water solution to 1500 times. With spraying water as a control. The results showed that: compared with water spraying, fruit russet was reduced in fruit expanding stage and turn-color stage, which could inhibit the accumulation of lignin, total phenol and total flavonoids, and affect the accumulation of eight phenolic acids in fruit peel. Spraying calcium fertilizer at fruit expanding stage was more conducive to inhibiting the accumulation of phenolic acids than that at turn stage, while spraying 1000 times of amino acid calcium at turn-color stage was more conducive to inhibiting the accumulation of gallic acid, caffeic acid, quercetin, coumaric acid and ferulic acid in pericarp than spraying 1500 times of water-soluble calcium and water. The results of this experiment can provide some guidance for grape production to supplement exogenous calcium and improve fruit quality.

1. Introduction
‘Shine Muscat’ is a diploid fresh grape variety [1]. After many years of experiments, the variety has good commercial quality, thin skin and crisp meat, natural rose fragrance, high sugar and low acid, and is deeply loved by consumers, with high planting efficiency. It is a hot variety for domestic development in the next few years [2]. However, ‘Shine Muscat’ grape is prone to fruit rust, which seriously affects its commercial and economic benefits [3]. At young fruit stage of grape, the cuticle is thin and the wax layer has not yet formed, so the protection ability is poor. When the fruit grows rapidly, the epidermal cells expand rapidly, which often leads to the cracking of the cuticle; or the cuticle is damaged due to the external bad factors, and the epidermal cells lose the protective barrier and are injured, resulting in the cell thrombosis and death, resulting in fruit russet [4]. However, in recent years, people pay more attention to the application of nitrogen, phosphorus and potassium fertilizer in grape production, but ignore the application of calcium fertilizer, which leads to the lack of calcium in grape fruit. Calcium is not only an essential nutrient for plant growth and development, but also calcium acts as a second messenger, which plays an important role in many physiological
processes. Calcium can maintain the structure of cell walls, maintain fruit firmness, reduce decay during storage, and control physiological diseases of fruit trees [5-6]. Because calcium is difficult to move in plant phloem, it is relatively less transported to fruit. In order to solve the problem of calcium deficiency of grape fruit and serious disease and quality decline caused by calcium deficiency, calcium fertilizer can be sprayed on the fruit before harvest. In this experiment, water-soluble calcium and amino acid calcium were sprayed on the leaves and ears of ‘Shine Muscat’ Grape during fruit expansion and color changing stages to study the effects of water-soluble calcium and amino acid calcium on the formation of grape fruit russet and phenolic substances, which has important practical significance for regulating the growth and development of grapes and improving fruit quality. The best calcium fertilizer treatment was selected through the experiment, which can provide guidance for efficient and high-quality production of grapes.

2. Materials and methods

2.1. Materials

Plant materials: In the vineyard of modern agricultural research and development base of Sichuan Agricultural University, the tree vigor is consistent, the soil, fertilizer and water management in the field is consistent, and there are no diseases and insect pests. The robust 5-year-old ‘Shine Muscat’ grape has a spacing of 1.5 m × 3.0 m, and 150 plants are planted per mu.

Fertilizer: Medium amount of element water-soluble calcium (calcium content ≥ 170 g · L⁻¹, the main component is calcium nitrate); Amino acid calcium water-soluble fertilizer (calcium content ≥ 30 g · L⁻¹, amino acid content ≥ 100 g · L⁻¹). They are all from Sichuan Guoguang agrochemical Co., Ltd.

2.2. Experimental design

The randomized block design was used in the experiment. The spraying concentration of amino acid calcium was set at 1000 times, and the spraying concentration of water-soluble calcium was set at 1500 times. The leaves and ears were sprayed on 30 days after full bloom (Fruit expansion stage) and 65 days after full bloom (Early stage of fruit color change), once every 10 days, for a total of 3 times. Water spray was used as control (ACK, BCK). Six treatments were set up in the experiment, each treatment was set with 5 grapes, 3 replicates, 3 trees as a replicate, a total of 90 grapes. After calcium fertilizer treatment, grape fruits were collected when they were ripe.

2.3. Test methods

2.3.1. Fruit russet index statistics. With reference to the method of Zhang Bo et al. [7], when sampling, the grading and rust rate statistics of each processed fruit were performed separately. According to the degree of 'fruit rust', the fruits were graded as follows: the rust-free fruit was graded as 0, the area of ‘fruit rust’ < 5% was graded as 1, 6% ~ 15% was graded as 2, 16% ~ 25% was graded as 3, and > 25% was graded as 4. Fruit rust index = (grade 0 × a +1 × b +2 × c+3 × d +4 × e) ×100/ (grade 4 × n). Note: a, b, c, d and e are the statistical number of 0-4 grade fruit respectively, and n is the total number of samples.

2.3.2. Determination of phenolic compounds. The contents of total phenols, total flavonoids, flavonols and flavanols were determined according to the method of Yan Juan et al. [8]. Gallic acid was used to make the standard curve, and gallic acid equivalent GAE·g⁻¹ FW was used to express the total phenol content; Rutin was used to make the standard curve, and rutin equivalent RE·g⁻¹ FW was used to express the total flavonoids content; Catechin was used to make the standard curve, and catechin equivalent CE·g⁻¹ FW was used to express the total flavanol content; Quercetin was used to make the standard curve, and quercetin equivalent QE·g⁻¹ FW was used to express the total flavonol content.
2.3.3. Determination method and chromatographic conditions of phenolic acid components. Sample pretreatment: Take out the frozen peel and pulp from the refrigerator at -80 °C, break them up and mix them well. Take the peel and pulp into the mortar respectively, add liquid nitrogen and grind them into powder, accurately weigh 0.5 g of mixed sample and put it into a 5 ml centrifuge tube, and add 1.5 ml of extraction solution [V (methanol) : V (water) : V (formic acid) = 70 : 28 : 2] under dark conditions to mix well. The centrifugation tube was put into the ultrasonic device to assist extraction for 30 min, then centrifuged at 4 °C and 5 000 R / min for 15 min. Take out the centrifuge tube and suck the supernatant. Filter with 0.45 μ m microporous membrane and store in refrigerator at -20℃ for analysis of phenolic acids. Chromatographic conditions: Agilent 1260 liquid chromatography, comatex C18 column (5 μ m, 46 mm × 250 mm), column temperature 30 ℃, injection volume 20 μ L. Mobile phase: A is 2% formic acid solution, B is acetonitrile, gradient elution, flow rate is 1 ml / min, elution procedure is shown in Table 1.

| Time | A%  | B%  |
|------|-----|-----|
| 0    | 95  | 5   |
| 25   | 85  | 15  |
| 42   | 78  | 22  |
| 50   | 64  | 36  |

2.4. Data processing
The test data were processed and plotted by Microsoft Excel 2010 and Sigma Plot software, and SPSS 20.0 software was used for statistical analysis.

3. Results and analysis

3.1. Statistics of fruit rust index and determination of lignin in ‘Shine Muscat’ grape under different calcium treatments
After different calcium treatments, the fruit rust index of different treatments was counted at the fruit ripening stage, and the content of lignin in peel was determined (Figure 1). The fruit rust index of spraying calcium fertilizer (A1, A2) was lower than that of spraying clear water (ACK) at fruit expansion stage, which were 15.60%, 16.80% and 23.50% respectively. However, the fruit rust index of 1000 times amino acid calcium (B1) was significantly lower than that of 1500 times water soluble calcium (B2) and clear water (BCK), and the fruit rust index of B2 and BCK were 38.50% and 49.70% respectively. The highest lignin content was 87.09 mg / g · DW in the peel of BCK, which was significantly higher than that of other treatments. The order of lignin content from top to bottom was BCK > B2 > ACK > B1 > A2 > A1. The lignin content of B2 treatment was significantly higher than that of B1, A1, A2 and ACK treatments. The lignin content of ACK was significantly higher than that of A1 and A2. There was no significant difference between A1 and A2. The results showed that spraying 1000 times of amino acid calcium and 1500 times of water-soluble calcium at fruit expanding stage and color changing stage could inhibit the accumulation of lignin compared with spraying water. There was no significant difference between the two calcium treatments at fruit expanding stage. However, the content of lignin after spraying 1000 times of amino acid calcium at color changing stage was significantly lower than that after spraying 1500 times of water-soluble calcium, indicating that spraying 1000 times of amino acid calcium at color changing stage inhibited the accumulation of lignin. The effect of this method is better.
Fig. 1 Statistics of fruit rust index and determination of lignin in ‘Shine Muscat’ grape under different calcium treatments

3.2. Effects of different calcium treatments on phenolic compounds in ‘Shine Muscat’ grape peel
As shown in Figure 2, BCK treatment had the highest total phenol content of 54.93 mg/g, which was significantly higher than other treatments. The total phenol content of the peel is BCK>B2>ACK>B1>A2 in descending order, B2 treatment was significantly higher than B1 treatment, and ACK treatment was significantly higher than A1 and A2 treatment. The content of total flavonoids in peel of BCK treatment was 5.05mg/g, which was significantly higher than that of other treatments, B2 treatment was significantly higher than that of B1 treatment, and ACK treatment was significantly higher than that of A1 and A2 treatment. There was no significant difference between BCK and B2, but it was significantly higher than other treatments. There was no significant difference between ACK and A1 and A2. B2 treatment has the lowest content of flavonols in the peel, which is only 1.46mg/g. The flavanol content of ‘Shine Muscat’ grape peel in BCK treatment was the highest, which was significantly higher than other treatments, but there was no significant difference among other treatments. The above results showed that the contents of total phenols, total flavonoids, flavonols and flavanols in BCK treatment were relatively high, indicating that the formation of fruit rust was related to the accumulation of phenols.

Fig.2 Effects of the phenolic compound of grape pericarp in different calcium treatments

3.3. Effects of different calcium treatments on phenolic acid content of grape pericarp
The eight kinds of phenolic acid content of gallic acid, epicatechin, chlorogenic acid, syringic acid, caffeic acid, quercitin, coumaric acid and ferulic acid in the pericarp of ‘Shine Muscat’ grape were
determined by HPLC under different calcium treatments. The results showed that there were certain differences in phenolic acid components under different calcium treatments (Table 2). The content of gallic acid in Pericarp of ‘Shine Muscat’ grape treated with BCK and B2 was relatively high, which was significantly higher than that of the other four treatments, but there was no significant difference among the other four treatments. Compared with A1, A2, ACK, B1 and B2, the gallic acid content of BCK increased by 17.36%, 17.35%, 28.46%, 19.89% and 2.23%, respectively. The content of chlorogenic acid in peel of BCK treatment was significantly higher than that of other treatments, and there was no significant difference among A2, ACK and B2 treatments. The order of chlorogenic acid content from high to low was BCK > B1 > ACK > A2 > B2 > A1. The contents of syringic acid and coumaric acid were not detected in A1, A2 and ACK treatments at mature stage. The content of eugenol in BCK treatment was significantly higher than that in B2 treatment, but there was no significant difference between BCK treatment and B1 treatment. The coumaric acid content of BCK treatment was significantly higher than that of B1 and B2 treatment, and there was no significant difference between B1 and B2 treatment.

The epicatechin content in the peel of B1 treatment was the highest, which was significantly higher than that of A1, A2 and ACK treatment, but there was no significant difference with that of B2 and BCK treatment. The caffeic acid content of BCK treatment was significantly higher than that of other treatments, and increased by 45.46%, 69.60%, 46.06%, 42.09% and 16.40% compared with A1, A2, ACK, B1 and B2 treatments, respectively. The content of ferulic acid in peel of B2 and BCK treatments was relatively high, which was significantly higher than that of the other four treatments. The order of ferulic acid content from high to low was B2 > BCK > B1 > A1 > A2 > ACK. The content of quercitrin in BCK treatment was significantly higher than that in other treatments, and there was no significant difference between B1 and B2 treatments. The order of ferulic acid content from high to low was BCK > B2 > B1 > A1 > ACK > A2. The results showed that the contents of gallic acid, epicatechin, chlorogenic acid, syringic acid, caffeic acid, quercetin, coumaric acid and ferulic acid in the pericarp of ‘Shine Muscat’ grape were affected by different calcium treatments, and the content of phenolic acid in BCK treatment was relatively high, which indicated that the accumulation of phenolic acid was related to the formation of russet.

| Treat - ment | Gallic Acid | Chlorogenic Acid | Syringic Acid | Epicatechin | Caffeic Acid | Ferulic Acid | Coumaric Acid | Quercitrin |
|-------------|-------------|-----------------|--------------|-------------|-------------|-------------|--------------|-----------|
| A1          | 6.02±0.07b  | 14.92±0.22d     | -            | 27.87±0.06b | 32.84±0.01c | 11.52±0.04c | -            | 6.49±0.01c |
| A2          | 6.02±0.12b  | 17.55±0.35c     | -            | 24.54±0.65c | 28.17±0.18d | 9.45±0.30d  | -            | 4.79±0.12e |
| ACK         | 5.50±0.04b  | 17.96±0.92c     | -            | 25.58±0.05c | 32.71±0.43c | 8.85±0.09d  | -            | 5.50±0.16d |
| B1          | 5.89±0.53b  | 22.79±0.39b     | 6.26±0.09ab  | 29.96±0.89a | 33.62±0.83c | 17.10±0.35b | 5.52±0.07b  | 8.83±0.79b |
| B2          | 6.91±0.29a  | 17.45±0.36c     | 6.50±0.16a   | 28.99±1.45ab| 41.04±2.01b | 18.57±0.87a | 5.80±0.20b  | 9.09±0.63b |
| BCK         | 7.06±0.11a  | 24.43±0.28a     | 6.08±0.15b   | 28.03±1.11ab| 47.77±1.12a | 18.43±0.29a | 6.82±0.12a  | 13.09±0.28a |

4. Conclusion

Different calcium treatments were applied to ‘Shine Muscat’ grape. The results showed that the fruit russet of ‘Shine Muscat’ grape was reduced by spraying calcium at fruit expanding stage and color changing stage compared with spraying water, which could inhibit the accumulation of lignin, total phenol and total flavonoids, and affect the accumulation of eight phenolic acids in the peel. Spraying calcium fertilizer at fruit expanding stage was more conducive to inhibiting the accumulation of phenolic acids than that at color changing stage, while spraying 1000 times of amino acid calcium at
color changing stage was more conducive to inhibiting the accumulation of gallic acid, caffeic acid, quercetin, coumaric acid and ferulic acid in pericarp than spraying 1500 times of water-soluble calcium and water.

Acknowledgements
This work was financially supported by the key scientific research projects for school-level discipline construction of Leshan normal university (LZD028).

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