Effects of calcium on gluconic acid accumulation in ‘Shine Muscat’ grape

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Abstract. Five year old ‘Shine Muscat’ grape was used as material to spray amino acid calcium and water-soluble calcium solution on grape leaves and ears during fruit expansion and color changing stages. The effects of exogenous calcium fertilizer on the formation of fruit rust and fruit quality of ‘Shine Muscat’ grape were studied in a randomized block design with 1000 times of amino acid calcium solution and 1500 times of water-soluble calcium solution, and the spraying water was used as the control. The results show that: compared with calcium fertilizer in fruit expanding stage, it is more beneficial to the accumulation of sugar components in pericarp to spraying calcium fertilizer in turn-color stage. The content of sugar in pulp can be increased by spraying calcium fertilizer compared with water in fruit expanding stage. Spraying calcium fertilizer in turn-color stage can promote the degradation of malic acid and citric acid in the pericarp of mature grape and inhibit the degradation of tartaric acid. Spraying calcium fertilizer in fruit expanding stage can promote the degradation of citric acid in mature grape pulp, while calcium amino acid can inhibit the degradation of oxalic acid and tartaric acid and promote the degradation of malic acid. Spraying calcium fertilizer in turn-color stage can promote the degradation of organic acids in mature grape pulp. Combined with the content of soluble sugar and titratable acid, spraying 1500 times of soluble calcium in fruit expansion period is beneficial to improve the fruit quality of ‘Shine Muscat’ grape.

1. Introduction
‘Shine Muscat’ grape is a diploid fresh variety [1]. It has good commercial quality, thin skin and crisp meat. It has natural rose flavor, high sugar and low acid. It is deeply loved by consumers and has high planting efficiency. It is a popular variety developed in China in recent years [2]. Calcium mainly exists in the form of calcium pectin in plants, which can deposit polysaccharides, maintain the strength of cell wall, enhance disease resistance, and play an important role in signal transduction and stress response [3]. Due to the slow effect of root application of calcium fertilizer, it is difficult to be absorbed and utilized by grapes, and foliar spraying has the advantages of fast effect and good effect, so foliar calcium fertilizer spraying in young fruit stage and expansion stage is the most common calcium supplement method in grape planting process [4]. At present, there are few reports about the effects of calcium fertilizer on the accumulation of gluconic acid and fruit quality of ‘Shine Muscat’. This test is to ‘Shine Muscat’ grape as test materials, spraying water-soluble calcium and amino acid calcium treatments on the leaves and ears of ‘Shine Muscat’ grapes during fruit expansion and color conversion periods to study their effects on the gluconic acid components and other fruit quality of ‘Shine Muscat’. Through experiments, the best calcium fertilizer treatment that can improve
the fruit quality of ‘Shine Muscat’ is selected, in order to provide a technical basis for the efficient and high-quality production of ‘Shine Muscat’ 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. Determination methods and chromatographic conditions of sugar and acid components. The extraction and determination of sugar and acid components in fruits refer to the methods of Hu Z.Q. et al. [5] and Chen et al. [6]. Determination method and chromatographic conditions of sugar components: The frozen pulp sample was ground into powder in liquid nitrogen, and 1.0g of sample powder was accurately weighed and 4mL of ultra-pure water was added, and the water bath was conducted at 80°C for 15min, 9 000 R • min⁻¹, centrifuged at 4°C for 15min, the supernatant was taken and transferred to a 10ml volumetric flask. The residue was added with 4ml ultrapure water, bathed at 80°C for 15min, centrifuged at 9000r•min⁻¹ at 4°C for 15min, and the supernatant was transferred to a 10ml volumetric flask. The volume of ultrapure water was fixed to 10ml. 1 ml sample solution was extracted with disposable syringe and filtered by 0.45 μm microporous membrane for injection analysis. Each sample was repeated 3 times. The chromatographic column was Thermo (4.6 mm × 250 mm) on a 0.5μm NH₂ column, and the detector was RID. The mobile phase was acetonitrile: water = 80:20 at the flow rate was 0.5 ml • min⁻¹, the column temperature was 30°C, the injection volume was 10 μL.

Determination method and chromatographic conditions of acid components: The frozen pulp sample was ground into powder in liquid nitrogen, 1.0 g of sample was accurately weighed, 4 ml of 0.2% phosphoric acid (pH = 2.6) was added to grind in ice bath; after ultrasonic treatment at room temperature for 20 min, centrifugation at 4°C and 10000 R • min⁻¹ for 15 min, the supernatant was transferred to a 10 ml volumetric flask; the residue was extracted and centrifuged again with 4 ml of 0.2% phosphoric acid, the supernatant was combined twice and the volume was fixed to 10 ml; After filtration by 0.45 μm microporous membrane, the filtrate was put into 1.5 ml injection bottle for standby. The chromatographic conditions for the determination of organic acids: C18 column (4.6 mm × 250 mm, 5 μm), mobile phase of 3% methanol: 97% 0.2% phosphoric acid water, flow rate of 0.8 ml • min⁻¹, detection wavelength of 210 nm, column temperature of 25°C, injection volume of 20 μL. The content was calculated according to the peak area of the sample and the standard curve of each organic acid.
2.3.2. Determination of other fruit quality indexes. When the fruit is ripe, take samples of the same size and no pests and diseases from all directions of the ears, quickly put them in the prepared ice box, and bring them back to the laboratory. A handheld sugar meter was used to determine the soluble solids content of grapes; The acid-base neutralization titration method was used to determine the titratable acid content [7]; The anthrone colorimetric method was used to determine the soluble sugar content [8].

2.4. Data processing
The test data were processed by Microsoft Excel 2016 and Sigma Plot software, and related charts were drawn. SPSS 26.0 software was used for statistical analysis. The significant level of difference between samples was 0.05.

3. Results and discussion

3.1. Effects of different calcium treatments on glucose components of ‘Shine Muscat’ grape
High performance liquid chromatography (HPLC) was used to determine the fructose, glucose and sucrose in the peel and flesh of ‘Shine Muscat’ grape at mature stage. The results showed that the content of fructose and glucose was the highest, while sucrose was the lowest. As shown in table 1, BCK treatment had the highest fructose content in ‘Shine Muscat’ grape peel at maturity, which was significantly higher than other treatments. There was no significant difference in fructose content between B1 and B2 treatments. The glucose content of B1, B2 and BCK treatments was relatively higher than that of A1, A2 and ACK treatments, but there was no significant difference among treatments. The sucrose content in the peel of B1 treatment was the highest, which was significantly higher than that of ACK, B2 and BCK treatment, but had no significant difference with A1 and A2 treatment. The total sugar content of B1, B2 and BCK treatments was relatively high, which was significantly higher than that of A1, A2 and ACK treatments, but there was no significant difference among treatments. The above results showed that the application of calcium fertilizer at the color changing stage was more conducive to the accumulation of sugar components in the pericarp than that at the fruit expanding stage, but the application of calcium fertilizer at the color changing stage had no significant effect on the accumulation of total sugar content in the pericarp than that at the fresh water stage.

| Treatment | Fructose(mg/g) | Glucose(mg/g) | Sucrose(mg/g) | Total sugar(mg/g) |
|-----------|---------------|---------------|---------------|-------------------|
| A1P       | 60.47±0.05d   | 71.48±0.89b   | 1.07±0.49ab   | 133.03±1.42d      |
| A2P       | 63.10±0.46c   | 72.46±0.70b   | 1.39±0.15ab   | 136.95±1.22c      |
| ACKP      | 55.65±0.05e   | 64.97±0.33c   | 0.77±0.29b    | 121.39±0.47e      |
| B1P       | 65.24±0.09b   | 75.50±0.57a   | 1.71±0.28a    | 142.45±0.66a      |
| B2P       | 64.75±0.27b   | 76.05±0.62a   | 0.92±0.06b    | 141.72±0.59a      |
| BCKP      | 66.41±0.49a   | 76.55±0.85a   | 0.95±0.22b    | 143.91±1.38a      |

As shown in table 2, the contents of fructose and glucose in ‘Shine Muscat’ grape pulp treated with BCK and B2 were relatively high, while the contents of fructose and total sugar in BCK and B2 were significantly higher than those in other treatments, and there was no significant difference between them. The contents of fructose, glucose and total sugar in A1 and A2 treatments were significantly higher than those in ACK treatment. The results showed that spraying 1000 times amino acid calcium and 1500 times water-soluble calcium in fruit expanding stage could increase the content of sugar components in flesh compared with spraying water, while spraying 1000 times amino acid calcium in fruit color changing stage could reduce the content of fructose, glucose and total sugar in flesh compared with spraying 1500 times water-soluble calcium and water.
Table 2 Effects of different calcium treatments on the sugar components of the ‘Shine Muscat’ grape flesh

| Treatment | Fructose (mg/g)      | Glucose (mg/g)     | Sucrose (mg/g) | Total sugar (mg/g) |
|-----------|----------------------|--------------------|----------------|--------------------|
| A1R       | 77.52±0.65c          | 93.05±2.90bc       | 0.52±0.27b     | 171.10±3.44bc      |
| A2R       | 77.48±0.03c          | 90.06±0.38d        | 0.50±0.15b     | 168.03±0.26c       |
| ACKR      | 71.29±0.01d          | 83.60±0.50e        | 0.49±0.14b     | 155.38±0.64d       |
| B1R       | 79.82±0.21b          | 92.00±0.49cd       | 0.39±0.16b     | 172.22±0.79b       |
| B2R       | 81.93±0.69a          | 95.99±0.19a        | 0.55±0.47b     | 178.47±1.25a       |
| BCKR      | 82.42±0.12a          | 95.05±0.19ab       | 1.45±0.53a     | 178.92±0.79a       |

3.2. Effects of different calcium treatments on organic acid content of ‘Shine Muscat’ grape

The four main components of organic acids (oxalic acid, tartaric acid, malic acid and citric acid) in grape skins and flesh of ‘Shine Muscat’ grape were determined by high performance liquid chromatography (HPLC). The accumulation of organic acids in grape skins and flesh of ‘Shine Muscat’ were different with different calcium treatments (Table 3). The content of oxalic acid in the peel of A2 treatment was significantly higher than that of other treatments. The content of tartaric acid in the peel of B1 and B2 treatments was significantly higher than that of other treatments. There was no significant difference. The contents of malic acid and citric acid in the peel of BCK treatment were significantly higher than other treatments. In terms of total sugar, the total acid content in the peel of BCK treatment was significantly higher than that of other treatments. Compared with treatments A1, A2, ACK, B1 and B2, they increased by 35.90%, 17.88%, 24.80%, 16.40% and 12.04% respectively. The above results indicate that spraying 1000 times amino acid calcium and 1500 times water-soluble calcium during the color conversion period can promote the degradation of malic acid and citric acid in the peel of ripe grapes, while inhibiting the degradation of tartaric acid.

Table 3 Effects of different calcium treatments on the organic acid component of the ‘Shine Muscat’ grape pericarp

| Treatment | Oxalic acid (mg/g) | Tartaric acid (mg/g) | Malic acid (mg/g) | Citric acid (mg/g) | Total acid (mg/g) |
|-----------|--------------------|----------------------|------------------|-------------------|------------------|
| A1P       | 0.056±0.008d       | 7.716±0.021d         | 4.699±0.044e     | 0.145±0.001f      | 12.615±0.029e    |
| A2P       | 0.138±0.000a       | 8.287±0.008c         | 5.959±0.010b     | 0.158±0.001d      | 14.542±0.012c    |
| ACKP      | 0.105±0.000b       | 7.821±0.004d         | 5.647±0.009c     | 0.163±0.000c      | 13.737±0.005d    |
| B1P       | 0.080±0.008c       | 9.122±0.009a         | 5.359±0.058d     | 0.167±0.000b      | 14.728±0.061c    |
| B2P       | 0.109±0.023b       | 9.030±0.169a         | 6.008±0.029b     | 0.154±0.001e      | 15.301±0.180b    |
| BCKP      | 0.093±0.001bc      | 8.562±0.016b         | 8.300±0.182a     | 0.188±0.000a      | 17.143±0.137a    |

As shown in table 4, the oxalic acid content in the pulp of ‘Shine Muscat’ grape treated with BCK was significantly higher than that of other treatments, and the tartaric acid content in the pulp treated with BCK and A1 was relatively higher than that of other four treatments. However, the contents of malic acid and total acid in the pulp of A2 and ACK treatments were relatively high, which were significantly higher than those of the other four treatments, and the content of citric acid in the pulp of ACK treatment was significantly higher than that of the other four treatments. To sum up, spraying amino acid calcium and water-soluble calcium at expansion stage and color changing stage can affect the content of oxalic acid, tartaric acid, malic acid and citric acid in grape pulp. Spraying different calcium fertilizers at color changing stage can promote the degradation of oxalic acid, tartaric acid, malic acid and citric acid in mature grape pulp, and B2 treatment has the best effect; Spraying two kinds of calcium fertilizer could promote the degradation of citric acid in mature grape pulp, while spraying amino acid calcium could inhibit the degradation of oxalic acid and tartaric acid, and promote the degradation of malic acid.
Table 4: Effects of different calcium treatments on the organic acid component of the 'Shine Muscat' grape flesh

| Treatment | oxalic acid (mg/g) | tartaric acid (mg/g) | malic acid (mg/g) | citric acid (mg/g) | total acid (mg/g) |
|-----------|--------------------|----------------------|-------------------|-------------------|-----------------|
| A1R       | 0.052±0.000b       | 3.546±0.005a         | 3.523±0.011c      | 0.124±0.002c      | 7.246±0.010c    |
| A2R       | 0.044±0.000c       | 3.255±0.003d         | 4.675±0.015a      | 0.128±0.000b      | 8.103±0.013a    |
| ACKR      | 0.042±0.000c       | 3.275±0.051d         | 4.674±0.014a      | 0.136±0.001a      | 8.127±0.041a    |
| B1R       | 0.045±0.005c       | 3.347±0.122d         | 3.500±0.058c      | 0.123±0.003c      | 7.014±0.147d    |
| B2R       | 0.045±0.004c       | 3.061±0.004c         | 3.255±0.024d      | 0.113±0.001d      | 6.473±0.018e    |
| BCKR      | 0.059±0.000a       | 3.562±0.003a         | 3.694±0.017b      | 0.131±0.000b      | 7.446±0.017b    |

3.3. Effects of different calcium treatments on other fruit quality of 'Shine Muscat' grape

It can be seen from Table 5 that after different calcium treatments, the soluble solid content of ‘Shine Muscat’ grape fruit was more than 18%, reaching the standard of high quality grape, and there was no significant difference in the soluble solid content among the treatments. The soluble sugar content of A2 treatment was significantly higher than that of A1 and ACK treatment, but there was no significant difference compared with B1, B2 and BCK treatment. The titratable acid content of A2 and ACK treatments was significantly lower than that of other treatments. In terms of solid-acid ratio and sugar-acid ratio, the solid-acid ratio and sugar-acid ratio of A2 treatment were relatively high. The results showed that 1500 times of water-soluble calcium could increase the soluble sugar content, reduce the titratable acid content and improve the fruit quality of ‘Shine Muscat’ grape.

Table 5: Effects of different calcium treatment on gluconic acid content of the ‘Shine Muscat’ grape

| Treatment | Soluble solids (%) | Soluble sugar (%) | Titratable acid (%) | Solid-acid ratio | Sugar-acid ratio |
|-----------|--------------------|-------------------|---------------------|------------------|-----------------|
| A1        | 18.13±1.85a        | 14.89±0.37 bc     | 0.30±0.01 a         | 60.59±7.41 b     | 49.68±2.20 b    |
| A2        | 18.57±1.23 a       | 15.90±0.31 a      | 0.25±0.02 b         | 75.73±1.02 a     | 65.06±3.16 a    |
| ACK       | 18.10±0.70 a       | 14.08±0.42 c      | 0.25±0.01 b         | 71.87±3.57 a     | 55.97±3.83 b    |
| B1        | 18.17±0.77 a       | 15.33±0.31 ab     | 0.30±0.03 a         | 61.04±3.22 b     | 53.36±3.91 b    |
| B2        | 18.07±0.68 a       | 15.71±0.69 ab     | 0.30±0.01 a         | 59.96±2.76 b     | 54.01±3.27 b    |
| BCK       | 19.27±0.88 a       | 16.24±0.34 a      | 0.32±0.01 a         | 60.58±1.72 b     | 51.10±1.53 b    |

4. Conclusion

Compared with calcium fertilizer in fruit expanding stage, it is more beneficial to the accumulation of sugar components in pericarp to spraying calcium fertilizer in turn-color stage. The content of sugar in pulp can be increased by spraying calcium fertilizer compared with water in fruit expanding stage. Spraying calcium fertilizer in turn-color stage can promote the degradation of malic acid and citric acid in the pericarp of mature grape and inhibit the degradation of tartaric acid. Spraying calcium fertilizer in fruit expanding stage can promote the degradation of citric acid in mature grape pulp, while calcium amino acid can inhibit the degradation of oxalic acid and tartaric acid and promote the degradation of malic acid. Spraying calcium fertilizer in turn-color stage can promote the degradation of organic acids in mature grape pulp. Combined with the content of soluble sugar and titratable acid, spraying 1500 times of soluble calcium in fruit expansion period is beneficial to improve the fruit quality of ‘Shine Muscat’ grape.

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References
[1] Matsumoto H. (2016) Effect of postharvest temperature on the muscat flavor and aroma volatile content in the berries of ‘Shine Muscat’, Postharvest Biology and Technology. 112.
[2] Bai M.D.(2019) Key Cultivation Techniques and Development Suggestions of Yunnan ‘Shine Muscat’ Grape, Fruit trees in southern China.48:116-121.
[3] Lu J.L, Zhang X.R, Xu Y.Z.(2011) Effects of Different Leaf Fertilizer on Grape Fruit Quality, North Horticulture.; 27-28.
[4] Yu H.L, Xu G.Y, Shen P.S.(2020) Technical Measures for Grape Calcium Supplement, Friends of Fruit Farmers, 34-43.
[5] Hu Z.Q, Wang H.C, Hu G.B.(2005) Determination of sugar, acid and vitamin C. Journal of Fruit Trees, 582-585.
[6] Chen M, Jiang Q, Yin X.R.,(2012) Effect of hot air treatment on organic acid- and sugar-metabolism in Ponkan (Citrus reticulata) fruit, Scientia Horticulturae, 147: 118-125.
[7] LI H.S.(2000) Principles and techniques of plant physiological and biochemical experiments, Beijing: Higher Education Press, 65-67.
[8] ZHANG S.W, ZONG Y.J, FANG C.Y.(2020) Optimization of rapid determination of soluble sugar content in barley leaves by anthrone colorimetry, Food Research and Development, 41: 196-200.