Effect of Magnesium Fertilizer on the Content of Sugar, Amino Acid and Protein in the Pulp and Pericarp of Magnesium-deficient Grapes

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Keywords: Magnesium Deficiency, Soil Application of Fertilizer, Foliar Application of Fertilizer, Sugar, Starch, Protein, Fruit Quality.

Abstract: Effects of magnesium fertilizer application on the contents of sugar, amino acid and protein in the pulp and pericarp of magnesium- deficient grapevines were studied under the field condition, in order to provide reference basis for formulating fertilization of grape. A field experiment with split plot design was conducted in which four plots of land set up in main district applied magnesium sulfate separately: 0kg/hm2, 112.5kg/hm2, 150kg/hm2, 187.5kg/hm2, while three blades were used in the deputy district to spray magnesium sulfate with different concentration: 0%, 0.2%, 0.4%. The results showed that the soluble sugar content in pulp of grapes was higher than that in pericarp, but the content of free amino acid and soluble protein was much lower than that in pericarp. The content of magnesium in grapes of magnesium-deficient grapevines was reduced, and the content of soluble sugar and soluble protein in grape pulp and pericarp was reduced, while the content of amino acid was accumulated. Proper application of magnesium can improve the content of magnesium in fruits, increase the content of soluble protein and soluble sugar in pulp and pericarp, and reduce the content of free amino acids. The treatment of S2F0.4 (i.e. 150kg/hm2 magnesium sulfate fertilizer applied in autumn, and 0.4% magnesium sulfate fertilizer sprayed on leaves in mid-May, mid-June and mid-July) has higher magnesium content in fruits and higher soluble protein and sugar content in pulp and pericarp, which is the best magnesium supplementation measure under this soil environment.

1 INTRODUCTION

Magnesium compared with other cations, plays an irreplaceable and important role in plant physiological action, in which it participates in photosynthesis, carbon and nitrogen metabolism and etc (Beale 1999, Wang 2004). In southern China, magnesium of soil is easy to be lost due to soil movement and leaching affected by climate, strong acidity of soil and other factors. As a result, the ability of the soil in magnesium application is reduced, and surely magnesium deficiency in crop is becoming increasingly serious (Guo 2010, Bai 2004). Grapevines have a large demand for magnesium, so magnesium deficiency possibly occurs all year round. If magnesium is deficient in grapevines, chloroplast structure will be changed, photosynthetic efficiency will be lowed and protein synthesis will be blocked, which will affect the absorption of other mineral elements and result in the reduction of fruit quality (Ma 2017, Yang 2012, Ma 2018). There are a lot of research on magnesium nutrition at home and abroad, but mostly concentrated on the influences of magnesium deficiency in plant photosynthesis, enzyme system and so on, and inclined to study potted vegetable and sand culture fruit tree seedlings (Xie 2009, Wu 2007). Under field conditions, research on the the aspect is relatively few that how soil application and foliar application of magnesium fertilizer in magnesium-deficient fruit trees influence the content of magnesium of the fruit and content of sugar, amino acid and protein in the pulp and pericarp. As a result, this paper is going to analyze significant difference in the magnesium content of the fruit and sugar, amino acid and protein content in the pulp and pericarp after applying and spraying magnesium

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fertilizer in magnesium-deficient grapevines in a vineyard, and evaluate the effect of soil application and foliar application of magnesium fertilizer on dry matter accumulation in grape pulp and pericarp, and analyze on the correlation between fruit magnesium content and sugar, amino acid and protein content in grape pulp and pericarp.

2 MATERIALS AND METHODS

2.1 Test Field Condition

The experimental site is located in Baosheng Village, Longquanyi District, Sichuan Province, China. It is a subtropical humid climate with mild climate, abundant rainfall and distinct seasons. The average annual sunshine is 1032.9h, with the most sunshine in August and the least sunshine in December. Annual average temperature is 16.5 °C, annual average frost-free period is 297d, and the average annual rainfall is 895.6 mm with annual average relative humidity of 81%. Through nutrient analysis on the soil and mature leaves of grapevines in the test site, it was found that the soil of test site was flat red sandy soil, with organic matter of 19.76g/kg, pH value of 5.56, nitrate nitrogen of 18.29mg/kg, ammonium nitrogen of 8.56mg/kg, available potassium of 104.34mg/kg, available phosphorus of 20.76mg/kg, available calcium of 342.35mg/kg, available magnesium of 40.79mg/kg, available manganese of 17.89mg/kg, available zinc of 1.23mg/kg and available iron of 34.78mg/kg. The element content in mature grape leaves were potassium 5.93g/kg, calcium 14.84g/kg, magnesium 1.6g/kg, iron 74.34mg/kg, manganese 48.36mg/kg, zinc 24.78mg/kg and copper 12.36mg/kg. According to the nutrition grading standards of grape leaves of Li Gangli (Liu 2006), it was found that the potassium, calcium, zinc, iron and copper elements in grape were all at moderate levels in this area, but the magnesium content was deficient seriously.

2.2 Design of Experiment

A field experiment with split plot design was conducted in which four plots of land set up in main district applied magnesium sulfate ($MgSO4·7H2O$) separately: 0kg/hm2, 112.5kg/hm2, 150kg/hm2, 187.5kg/hm2, and they were represented by S0, S1, S2, and S3. In the deputy district three concentrations of foliar spraying of 0%, 0.2%, 0.4% were applied and represented by F0.2, F0.2, F0.4. A total of 12 treatments were tested, which were successively S0F0, S0F0.2, S0F0.4, S1F0, S1F0, S1F0, S2F0, S2F0, S2F0, S3F0, S3F0, S3F0 and S3F0. Grapevines were evenly selected from each plot, and repeatedly selected for three times to get 360 grapevines. Magnesium sulfate was applied in the soil with the base fertilizer in autumn, and then rotated till. Foliar application can be divided into three times, respectively in mid-May, mid-June and mid-July, with water dripping in the leaves as the limit. The experiment has been running for two consecutive years since October 2014.

2.3 Measuring Objects and Methods

At the ripening stage, 10 brunch of grapes were randomly selected for each treatment, and the fruits at the upper, middle, lower, east, west, south and north locations were picked for each brunch. Half of the fruits were dried, ground and screened after washing for determination of magnesium content. After separating the pericarp and pulp of the other half of the fruits, the pericarp and pulp were quickly frozen with liquid nitrogen and stored at -20℃ for determination of sugars, amino acids and proteins in the pericarp and pulp. The determination methods were as follows:

1.3.1 Determination of magnesium content in fruit. According to the standard set by China Agricultural University, (1) the leaves should be washed for 30s with 0.1mol/L hydrochloric acid solution;(2) 0.1% detergent for 30s; (3) Remove and rinse with running water; (4) Clean with ionized water and remove surface moisture with filter paper. The washed fruits were placed in an oven at 105℃ to kill enzymes for 20min, and then dried at 70-80℃. After grinding with a stainless steel plant grinder, pass a 0.25mm aperture sieve (60 mesh) and store in a dryer for testing. Magnesium of fruit was burned in a muff furnace at 550℃ to make ash, dissolved in dilute hydrochloric acid, and determined by flame atomic absorption spectrometry (Wu 2003).

1.3.2 Determination of sugars, amino acids and proteins in the pericarp and pulp of fruit. The pericarp and pulp were ground into powder under liquid nitrogen respectively, and then related determination was conducted. The vitamin C was determined by dichlorophenol indophenol titration [10]. Soluble sugar content was determined by anthrone colorimetric method (Xiong 2003). Invert sugar, reducing sugar, sucrose and total sugar were determined by film reagent titration (Huang 2009). Free amino acids were determined by ninhydrin (Hao 2014). Soluble protein was determined by

Coomassie bright blue G-250 method, and bovine serum protein was used as the standard curve (Hao 2014).

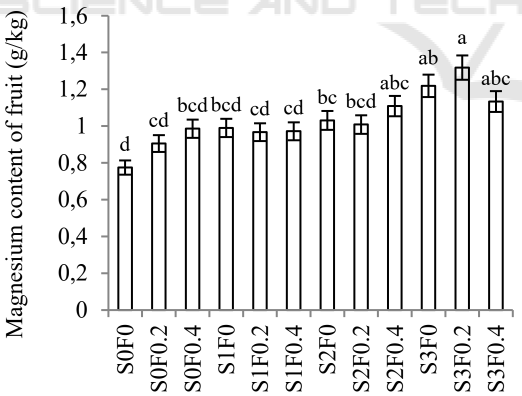
2.4 Data Analysis and Processing

The test data were processed and plotted by Microsoft Excel 2010 software, and SPSS18.0 software was used for statistical analysis.

3 RESULTS AND ANALYSIS

3.1 Effect of Magnesium Fertilizer Application on Magnesium Content in Magnesium-deficient Grapes

Based on Figure 1, after magnesium supplementation, the magnesium content in fruits increased significantly, and the magnesium content in S3F0.2 fruits was the highest, reaching 1.318g/kg. But there was no significant difference with that in S3F0.4, S3F0 and S2F0. Table 1 shows the effect of two kinds of magnesium application on magnesium content in fruits, and it is found that soil application can significantly increase magnesium content in fruits, while foliar application had no significant effect on magnesium content. And the interaction effect between the two was not significant.



Note: different letters in the figure indicate significant difference between different treatments at 0.05 level.

Figure 1: Effects of Magnesium Supplementation on Magnesium Content in Magnesium-deficient Grape Leaves.

Table 1: Effect Appraisal of Soil and Foliar Application of Magnesium Fertilizer on Soluble Sugar Content in Pericarp and Pulp of Magnesium-deficient Chlorosis Grape.

Variance analysis F value	Soil application (S)	Foliar application(F)	S×F
Magnesium content	12.08**	0.58	1.34

Note: ** indicated that F test reached a very significant level (P < 0.01), * indicated that F test reached a significant level (P < 0.05). The following is the same.

3.2 Effects of Magnesium Supplementation on Soluble Sugar Content in Pulp and Pericarp of Magnesium-deficient Grapes

It can be seen from Figure 2-3 that the soluble sugar content in pulp was higher than that in pericarp. The soluble sugar content in pulp and pericarp of S0F0 without magnesium fertilizer application was the lowest with only 10.03% in pulp and 7.52% in pericarp. After applying magnesium fertilizer, the soluble sugar content in pulp and pericarp increased significantly. The higher the amount of magnesium applied in 0-150kg/hm2, the higher the soluble sugar content was in pulp and pericarp. The highest soluble sugar content in pulp of S3F0 was 12.58%, which was not significantly different from that of S2F0.4, but significantly higher than that of other treatments, while the soluble sugar content in pericarp of S2F0.4 was the highest, reaching 11.02%, which was not significantly different from S3F0 and S1F0.4. While the soluble sugar content in pulp and pericarp decreased significantly. When the soil application amount was 187.5 kg/hm2, and the higher the leaf concentration was, the lower the sugar content was. As can be seen from Table 2, soil application of magnesium fertilizer could significantly affect the soluble sugar content in pulp and pericarp, while foliar application of magnesium fertilizer had no significant effect on the soluble sugar content of pulp and pericarp, and the interaction between the two was not significant.

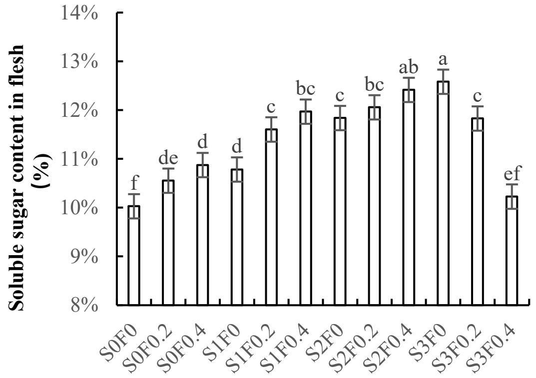


Figure 2: Effects of Magnesium Supplementation on Soluble Sugar Content in flesh of Magnesium-deficient Grape.

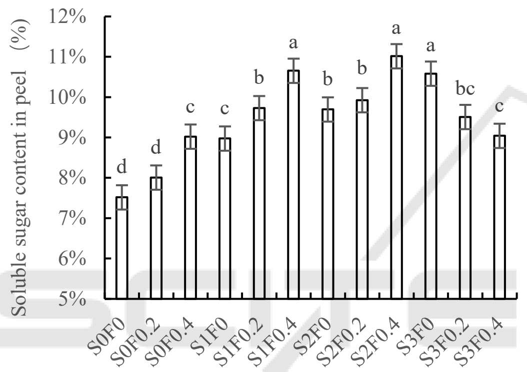


Figure 3: Effects of Magnesium Supplementation on Soluble Sugar Content in Pulp of Magnesium-deficient Grape.

Table 2: Effect Appraisal of Soil Application and Foliar application of Magnesium Fertilizer on Soluble Sugar Content in Pericarp and Pulp of Magnesium-deficient Chlorosis Grape.

Variance analysis F value	Soil application (S)	Foliar application(F)	S×F
Soluble sugar content in pulp	46.80**	1.49	23.49**
Soluble sugar content in pericarp	58.99**	16.12**	14.70**

3.3 Effects of Magnesium Supplementation on Free Amino Acid Content of Pulp and Pericarp in Pulp and Pericarp of Magnesium-deficient Grape

As can be seen from Figure 4-5, free amino acid content in the pulp was relatively less than that in pericarp, and free amino acid contentin pulp and pericarp was the highest in S0F0 without magnesium fertilizer application, reaching 42.26 mg / 100 g in pulp, which had no significant difference from S0F0. 2, S0F0. 4, S1F0, S1F0. 2 and S1F0. 4, but was significantly higher than other treatments. Free amino acid content in the pericarp was 104.52 mg / 100 g, which had no significant difference from S0F0. 2, but was significantly higher than other treatments. After the application of magnesium fertilizer, the free amino acid content in the pulp and pericarp was significantly reduced. The free amino acid content in the pulp and pericarp of S2F0.4 was the lowest, only 28.40mg/100g in the pulp, which was not significantly different from that of S3F0 but was significantly lower than that of other treatments. From Table 3, the effect appraisal of soil application and foliar application of magnesium fertilizer on free amino acid content in pulp and pericarp of magnesium-deficient chlorosis grape, it is seen that soil application of magnesium fertilizer can significantly affect free amino acid content in the pulp and pericarp, while the effect of foliar application of magnesium fertilizer on free amino acid content in pulp was not significant but on the pericarp. No significant interaction on free amino acid content in pericarp between soil application and foliar application of magnesium fertilizer is showed, but showed in pulp.

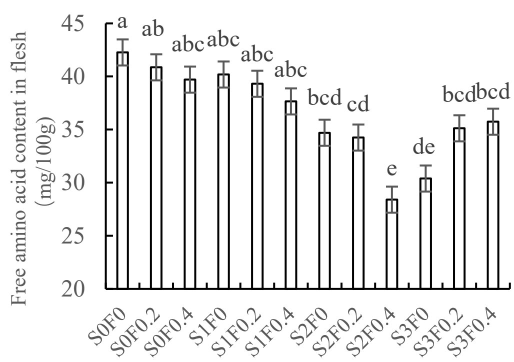


Figure 4: Effect of Magnesium Supplementation on Free amino Acid Content in Pericarp of Magnesium-deficient Grape.

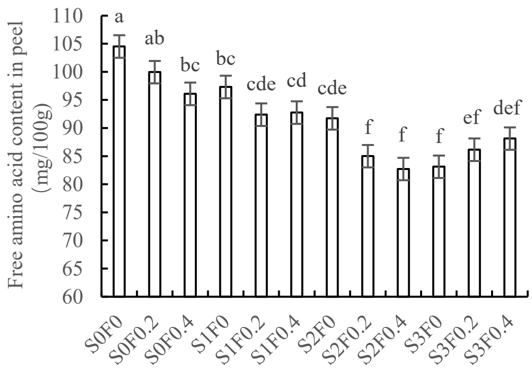


Figure 5: Effect of Magnesium Supplementation on Free amino Acid Content in Pulp of Magnesium-deficient Grape.

Table 3: Effect Appraisal of Soil Application and Foliar Application of Magnesium Fertilizer on Free Amino Acid Content in Pulp and Pericarp of Magnesium-deficient Chlorosis Grape.

Variance analysis F value	Soil application (S)	Foliar application(F)	S×F
Free amino acid content in pulp	14.46**	1.26	1.91
Free amino acid content in pericarp	34.63**	4.92*	2.79*

3.4 Effects of Magnesium Supplementation on Soluble Protein in Pericarp and Pulp of Magnesium-deficient Grape

Figure 6-7 shows the effect of magnesium application on soluble protein content in pericarp and pulp of magnesium-deficient grape. It can be seen that the soluble protein content in the pericarp was higher than that in the pulp, which was 2-3 times of the soluble protein content of pulp on average. S0F0 without magnesium fertilizer application was the lowest soluble protein content in the pulp and pericarp, only 0.63 mg g-1FW in the pulp and 1.71mg g-1FW in the pericarp. After applying magnesium fertilizer, the content of soluble protein in pulp and pericarp increased significantly. When the soil application was 0-150kg/hm2, the higher the magnesium fertilizer application was, the higher the soluble protein content was in the pulp and pericarp. The content of soluble protein in the pulp and pericarp of S2F0.4 was the highest, reaching 0.90mg g-1FW in the pulp. However, the difference between S2F0.4 and S2F0.2 and S3F0 was not significant, but was significantly higher than that of other treatments. And it was 2.47mg-g-1FW in the pericarp, and not significantly different from S2F0.2 and S3F0. When the soil application was 187.5 kg/hm2, the soluble protein content of pulp and pericarp was significantly reduced. And the higher the leaf concentration was, the lower the protein content was. Table 4 shows effect appraisal of soil application and foliar application of magnesium fertilizer magnesium fertilizer on soluble protein content content in pulp and pericarp of magnesium-deficient chlorosis grape. It can be seen that soil application of magnesium fertilizer can significantly affect the soluble protein content in pulp, but the effect of foliar application of magnesium fertilize is not significant. There was no significant interaction between the two. Soil application and foliar application have a significant effect on the soluble protein content of the pericarp, and have a very significant interaction.

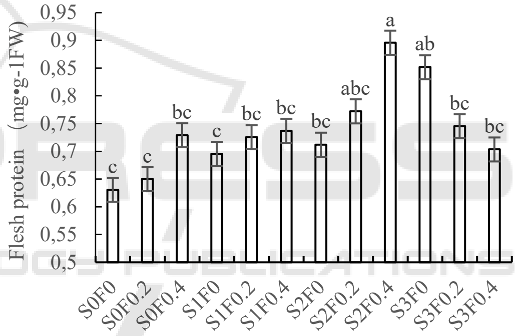


Figure 6: Effects of Magnesium Supplementation on Soluble Protein Content in Pericarp of Magnesium-deficient Grape.

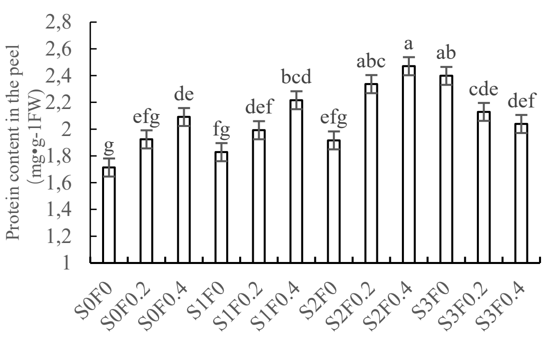


Figure 7: Effects of Magnesium Supplementation on Soluble Protein Content in Pulp of Magnesium-deficient Grape.

Table 4: Effect Appraisal of Soil Application and Foliar Application of Magnesium Fertilizer on Soluble Protein Content in Pulp and Pericarp of Magnesium-deficient Chlorosis Grape.

Variance analysis F value	Soil application (S)	Foliar application(F)	S×F
Soluble protein content in pulp	4.30*	1.22	2.47
Soluble protein content in pericarp	34.93**	17.82**	10.76**

3.5 Correlation Analysis between Magnesium Content in Grape Fruits and Content of Sugar, Amino Acid, Protein in Pulp and Pericarp of Grape after Magnesium Supplementation

As can be seen from Table 5, after magnesium fertilizer application, magnesium content in the grape was significantly positively correlated with the soluble sugar content in pulp and pericarp, significantly negatively correlated with the free amino acids content in pulp and pericarp, positively correlated with the soluble protein content in pulp, but had no significant correlation with the soluble protein content in the pericarp.

Table 5: Correlation analysis between magnesium content in grape fruits and sugar, amino acid, protein content in pulp and pericarp of grape.

correlation	Magnesium in the fruits
soluble sugar content of pulp	0.18**
soluble sugar content of pericarp	0.47**
free amino acid of pulp	-0.39**
free amino acid of pericarp	-0.55**
soluble protein content of pulp	0.39*
soluble protein content of pericarp	0.32

4 DISCUSSION

The application of magnesium in grape production is mainly based on soil application and liar spraying. In soils with magnesium deficiency, the effect of magnesium as base fertilizer is better. However, soil application of magnesium fertilizer is easily affected by soil acidity, soil texture, soil colloid type and other elements (especially exchangeable calcium and aluminum). The application of magnesium fertilizer on the leaves has the advantages of low dosage, low cost and quick effect, but it cannot fundamentally solve the symptoms of magnesium deficiency (Wen 2015, Wu 2013). In this experiment, it was found that soil application of magnesium fertilizer significantly increased the magnesium content in fruits, while liar spraying had no significant influence on the magnesium content in fruits. However, when soil application combined with liar spraying, there was a significant interaction effect on the increase of magnesium content in leaves.

Magnesium, the center of the chlorophyll element and enzyme activator, has important effect to photosynthesis. Its lack of can reduce the plant photosynthetic product, as a result, fruit quality drops. this study found that soluble sugar content is higher than that in pericarp. Moderate application of magnesium fertilizer can significantly improve the soluble sugar content of soil in pulp and pericarp. Magnesium fertilizer application can significantly improve sugar content in pulp and pericarp, while the effect of foliar spraying magnesium fertilizer to improve the fruit sugar content was not significant, but the effect of sugar content improvement is remarkable. Magnesium content in fruit and soluble sugar content in pulp and pericarp is very significant positive correlation. This is consistent with the results of Wen Mingxia (Wen 2015) et al., that magnesium application can significantly promote the transportation of magnesium to fruits and improve the magnesium content and soluble sugar content of fruits.

The effect of amino acids on plant nutrients is not only to supply nitrogen source, but more importantly, it has a great effect on plant physiological metabolism. Ding Yuchuan et al. (Ding 2009) found that Mg^{2+} concentration was beneficial to enhance the ability of the root system to synthesize amino acids, while low Mg^{2+} and high Mg^{2+} concentration inhibited the ability of the root system to synthesize amino acids to a certain extent. But this study found that free amino acid content in pulp and pericarp of magnesium-deficient grape increased, and after appropriate application of magnesium fertilizer, the free amino acid content decreases. The magnesium content t and free amino acid content in fruit is very significant negative correlation, which is in accordance with the study of Li Yan et al. (Li 2001) that the total amount of free amino acids in longan leaves increased under the stress of magnesium deficiency, and is inconsistent with the study of Zhu Yongxing et al. (Zhu 2003) that amino acids of tea leaves increased after spraying magnesium fertilizer. The reasons for these differences may be due to the magnesium deficiency stress under different crop types and different parts of the amino acid content, or because the amino acid differences between species.

As a component of ribosome, magnesium can stabilize the necessary ribosome configuration for protein synthesis, and magnesium deficiency leads to the dissociation of ribosome into small ribosome subunits (Menge 1982). In this experiment, it was found that when magnesium deficiency occurred, protein content in grape pulp and pericarp decreased, which was consistent with the research results of Yan Li (Li 2001) et al. that protein synthesis in longan leaves was hindered and decomposition was intensified under the stress of magnesium deficiency. After application of magnesium fertilizer, soluble protein content in pulp and pericarp reduces after rising, which indicates that a moderate amount of supplementary magnesium fertilizer can effectively increase protein content of the magnesium-deficient grapes, but excess is suppressed. Magnesium content in fruit and protein in pulp have significant positive correlation, which is consistent with the results of magnesium fertilizer application on tobacco (Li 2001), balsam pear (Sun 2010, Lin 2015), watermelon and so on.

5 CONCLUSIONS

The content of soluble sugar in pulp is higher than that in pericarp, but the content of free amino acid and soluble protein is much lower than that in pericarp. When magnesium is deficient, the content of magnesium in grape decreases, the content of soluble sugar and soluble protein in pulp and pericarp decreases, but the content of amino acid accumulates. Appropriate application of magnesium fertilizer can improve magnesium content in fruits, increase soluble protein and soluble sugar content in pulp and pericarp, and reduce free amino acid content. The fruits of S2F0.4 (i.e., 150kg/hm2 magnesium sulfate fertilizer applied in autumn, and 0.4% magnesium sulfate fertilizer sprayed on the leaves in mid-may, mid-June and mid-July) have high magnesium content and high soluble protein and sugar content in the pulp and pericarp, which is the best magnesium supplementation measure in this soil environment.

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