Effect of Spraying Brassinolide on Fruit Quality of *Citrus grandis* cv. 'Huangjinmiyou' and 'Hongroumiyou'

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**Abstract.** Brassinolide (BR) is one of the new green and environmental-friendly plant growth regulators. 'Huangjinmiyou' and 'Hongroumiyou' are mutated from *Citrus grandis* cv. 'Guanximiyou' including rich nutritional ingredients. When they are introduced into Sichuan Province, the fruits showed lower sugar accumulation and higher acid content than that in Fujian Province. We compared the fruit quality by spraying 0.02, 0.04, and 0.06 mg L⁻¹ of brassinolide to improve the fruit quality of pummelo production in Sichuan Province. The results showed that spraying approximate concentration of brassinolide could increase the flesh weight and edible rate, and ease granulation index of 'Huangjinmiyou' and 'Hongroumiyou'. Also, brassinolide could significantly promote the accumulation of total soluble solid, vitamin C and total sugar. The TSS-acid ratio continuously increased with the increase of brassinolide, obviously improving the taste quality. The best effect on the improvement of fruit quality was selected by 0.06 mg L⁻¹ of brassinolide. This study will provide related basis for high-quality and high-efficient production of pummelo cultivars in Sichuan Province.

1. Introduction

Brassinolide (BR) is one of the new green and environmental-friendly plant growth regulators, isolated from *Brassica napus* pollen [1]. It has been reported that appropriate concentration of brassinolide spraying on the leaf and seed can improve crop growth and fruit quality, ease the suffering from insect pests and diseases damage, as well as promote the cold and drought resistance of crops [2]. Spraying 0.08 to 0.12 mg L⁻¹ of brassinolide could improve the growth of maize [3]. Zhou et al. [4] suggested that brassinolide could increase the seed kernel oil yield of *Camellia camellia*.

Pummelo [*Citrus grandis* (L.) osbeck], belonging to genus *Citrus* L., family Rutaceae, is a commercially valuable fruit crop [5]. China is one of major centers of origination and genetic diversity, which possesses abundant pummelo germplasms. As an important fruit crop, pummelo has been domesticated and cultivated for more than 3000 years [6], including three major production areas, Southeast coastal, South China and Southwest China [7]. Until now, over 200 pummelo varieties have been selected and cultivated in China [8]. 'Huangjinmiyou' [9] and 'Hongroumiyou' [10] are mutated from 'Guanximiyou', which have been widely cultivated in Fujian and Sichuan Provinces. Their fruits contain rich nutritional ingredients and trace elements such as vitamins, antioxidant compounds for healthy diets.
Due to the lower annual average temperature and less mean annual sunshine durations, the mature fruits of ‘Huangjinmiyou’ and ‘Hongroumiyou’ introduced into Sichuan Province showed lower sugar accumulation and higher acid content than that in Fujian Province. Previous studies suggested that the fruit quality could be improved by spraying brassinolide, such as strawberry, citrus, and grape [11]. In this study, we compared the fruit quality by spraying different concentration of brassinolide to better provide guidance for high-quality and high-efficient production of pummelo in Sichuan Province.

2. Materials and methods

2.1. Plant materials

Two pummelo cultivars, *C. grandis* cv. ‘Huangjinmiyou’ and ‘Hongroumiyou’ rootstocked by wild *C. grandis* were used in this study. The experiments were carried out in the orchard in Pujiang County, Chengdu, Sichuan Province. 0.02, 0.04, and 0.06 mg•L⁻¹ of brassinolide was sprayed around the crown of ‘Huangjinmiyou’ and ‘Hongroumiyou’ at 98, 117, 137 and 158 days after flowering. Spraying water was used as the control check. Three trees were treated for each treatment with three repeats per treatment.

2.2. Fruit quality determination

Vernier caliper was used to detect the peel thickness, longitudinal and transverse diameter of fruits. The weight per fruit and flesh weight was measured by an electronic balance. According to these data, fruit shape index and edible rate were calculated. Granulation index = (a₁ + 2a₂) / [2 × (a₀ + a₁ + a₂)], where a₀, a₁, and a₂ indicate the number of segment without granulation, the number of segment with less than half of granulation, and the number of segment with more than half of granulation, respectively.

Chromatograph (CM-2500d/CM-2600d, KONICA MINOLTA, Japan) was used to detect the fruit coloration, including $L^*$, $a^*$, $b^*$, $C^*$, $h^o$ [12]. The value of $L^*$, $a^*$, $b^*$, $C^*$ and $h^o$ represent the lightness (0 ~ 100), redness (-60 ~ +60), yellowness (-60 ~ +60), chroma, and hug angle, respectively. The value of $C^*$ and $h^o$ was calculated by $(a^*^2 + b^*^2)^{1/2}$ and arctan ($b^*/a^*$).

The total soluble solid (TSS) content was detected by the hand-held refractometer. 2,6-dichloroindophenol titration method was used to measure vitamin C content [13]. Total sugar and acid was examined by using anthrone colorimetric [14] and acid-base neutralization method [14], respectively. The sugar contents were calculated according to Xiong et al. [15]: Reducing sugar = D/V₁ × 1000, Invert sugar = D/V₂ × 1000, Sucrose = (invert sugar − reducing sugar) × 0.95, Total sugar = reducing sugar + sucrose, where D, V₁, and V₂ represent the glucose content corresponding to 10 mL of Fehling reagent, titration volume of reducing sugar solution and invert sugar solution, respectively.

2.3. Data analysis

Significant differences between the means of the treatments were determined with 95% confidence (p < 0.05) limit by Duncan multiple range test using SPSS18.0 (IBM, USA). Data are shown as the means of three replicates.

3. Results

3.1. External fruit quality

The external fruit quality of two pummelo cultivars by spraying brassinolide was shown in the Table 1. The peel thickness revealed no significant differences among different concentrations. Spraying 0.06 mg•L⁻¹ of brassinolide significantly increased the transverse diameter with the value of 18.30 cm for ‘Huangjinmiyou’ and 17.30 cm for ‘Hongroumiyou’. Obvious change trends were observed in longitudinal diameter by spraying different concentration of brassinolide. So did in fruit shape index. Compared with the control group, 0.02 mg•L⁻¹ of brassinolide slightly decreased the weight per fruit,
while 0.04 and 0.06 mg•L\(^{-1}\) of brassinolide significantly increased average fruit weight with the maximum of 2.039 kg for 'Huangjinmiyou' and 1.653 kg for 'Hongroumiyou'. Similarly, spraying brassinolide had different level of increasing effects on the flesh weight. The maximum edible rate reached 84.94% and 81.27% when spraying 0.06 mg•L\(^{-1}\) of brassinolide, much higher than other treatments. There were no significant differences for the segments among different treatments. The control group showed the maximum granulation index of 0.60 and 0.33, significantly higher than the treatments. When we sprayed 0.06 mg•L\(^{-1}\) of brassinolide, the granulation index decreased to the minimum value of 0.31 and 0.08 for 'Huangjinmiyou' and 'Hongroumiyou', respectively. Therefore, spraying 0.06 mg•L\(^{-1}\) of brassinolide significantly improved the external fruit quality of two pummelo cultivars.

Table 1. Effect of spraying brassinolide on external fruit quality of *Citrus grandis* cv. 'Huangjinmiyou' and 'Hongroumiyou'

| Cultivar       | Brassinolide concentration /mg•L\(^{-1}\) | Peel thickness/cm | Longitudinal diameter/cm | Transverse diameter/cm | Fruit shape index | Fruit weight/kg | Flesh weight/kg | Edible rate/% | Segment Granulation index |
|----------------|---------------------------------------------|-------------------|--------------------------|------------------------|------------------|----------------|----------------|---------------|------------------------|
|                | 0.02                                        | 1.20a             | 15.90b                   | 16.30c                 | 0.98a            | 1.620c         | 1.323c         | 81.49b        | 15.30a                  |
| 'Huangjinmiyou' | 0.04                                        | 1.20a             | 17.20a                   | 17.00b                 | 1.01a            | 1.844b         | 1.525b         | 82.75b        | 14.70a                  |
|                | 0.06                                        | 1.10a             | 16.80a                   | 18.30a                 | 0.92a            | 2.039a         | 1.735a         | 84.94a        | 15.00a                  |
| 'Hongroumiyou'  | 0.02                                        | 1.60a             | 15.40b                   | 16.10b                 | 0.96a            | 1.292c         | 0.997d         | 77.17b        | 14.30a                  |
|                | 0.04                                        | 1.50a             | 17.70a                   | 17.20a                 | 1.04a            | 1.616a         | 1.262b         | 78.23b        | 14.70a                  |
|                | 0.06                                        | 1.50a             | 15.60b                   | 17.30a                 | 0.97a            | 1.653a         | 1.345a         | 81.27a        | 14.70a                  |
| CK             | 0.02                                        | 1.70a             | 17.30a                   | 15.90b                 | 1.09a            | 1.565b         | 1.183c         | 75.50c        | 14.30a                  |
| 'Hongroumiyou'  | 0.04                                        | 1.70a             | 17.30a                   | 15.90b                 | 1.09a            | 1.565b         | 1.183c         | 75.50c        | 14.30a                  |
|                | 0.06                                        | 1.70a             | 17.30a                   | 15.90b                 | 1.09a            | 1.565b         | 1.183c         | 75.50c        | 14.30a                  |

Note: The different normal letters in rows indicate significant difference at 0.05 level. The same as below.

3.2. The fruit chromatic aberration

As shown in the Table 2, the effect on flesh chromatism of brassinolide was evaluated. \(L^*\) value among different treatments was all higher than the CK, with the maximum value of 54.90 for 'Huangjinmiyou' and 53.22 for 'Hongroumiyou' by spraying 0.06 mg•L\(^{-1}\) of brassinolide. The minimum value of \(a^*\) was 9.95 and 6.48 exhibited in the CK. Spraying brassinolide obviously increased \(a^*\) value, which showed significantly effect at 0.04 and 0.06 mg•L\(^{-1}\) of brassinolide, with 11.24 and 8.99, respectively. There were no obvious decreases for \(b^*\) value by spraying brassinolide. \(C^*\) value reached the maximum of 21.17 and 10.62 by spraying 0.06 mg•L\(^{-1}\) of brassinolide, but no significant differences were observed among different concentrations. The remaining parameter, \(h^o\), showed an increase trend for 'Huangjinmiyou' and a decrease change for 'Hongroumiyou' with the increasing concentration of brassinolide. The maximum \(h^o\) value of 1.27 and 0.72 exhibited at 0.06 mg•L\(^{-1}\) of brassinolide and control group. After treatment by brassinolide, the flesh color of 'Hongroumiyou' turned light red color. This difference among parameters might be related to the different color of 'Huangjinmiyou' and 'Hongroumiyou'.

Table 2. Effect of spraying brassinolide on flesh chromatism of *Citrus grandis* cv. 'Huangjinmiyou' and 'Hongroumiyou'

| Cultivar       | Brassinolide concentration /mg•L\(^{-1}\) | \(L^*\) | \(a^*\) | \(b^*\) | \(C^*\) | \(h^o\) |
|----------------|---------------------------------------------|-------|-------|-------|-------|-------|
|                | 0.02                                        | 1.20a | 9.95a | 6.48a | 21.17 | 1.27  |
| 'Huangjinmiyou' | 0.04                                        | 1.20a | 9.95a | 6.48a | 21.17 | 1.27  |
|                | 0.06                                        | 1.20a | 9.95a | 6.48a | 21.17 | 1.27  |
| CK             | 0.02                                        | 1.70a | 9.95a | 6.48a | 21.17 | 1.27  |
| 'Hongroumiyou'  | 0.04                                        | 1.70a | 9.95a | 6.48a | 21.17 | 1.27  |
|                | 0.06                                        | 1.70a | 9.95a | 6.48a | 21.17 | 1.27  |
| CK             | 0.02                                        | 1.70a | 9.95a | 6.48a | 21.17 | 1.27  |
Table 2. Effect of spraying brassinolide on flesh chromatism of *Citrus grandis* cv. 'Huangjinmiyou' and 'Hongroumiyou'

| Cultivar          | Brassinolide concentration/mg•L⁻¹ | L*      | a*      | b*      | C°     | b°     |
|-------------------|-----------------------------------|---------|---------|---------|--------|--------|
| 'Huangjinmiyou'   | 0.02                              | 49.59±2.62b | 10.86±2.97b | 18.50±2.56a | 21.45±1.76a | 1.04±0.02c |
|                   | 0.04                              | 49.79±2.03b | 11.24±3.12a | 18.21±1.35a | 21.40±2.67a | 1.12±0.01b |
|                   | 0.06                              | 54.90±3.15a | 10.21±2.19b | 18.54±3.56a | 21.17±2.06a | 1.27±0.01a |
| CK                |                                   | 48.58±5.14b | 9.95±2.03c  | 16.07±1.98b | 18.90±2.56b | 1.02±0.02c |
| 'Hongroumiyou'    | 0.02                              | 47.33±2.68c | 6.92±2.42b  | 5.91±1.76a  | 9.10±1.78b  | 0.71±0.01a  |
|                   | 0.04                              | 51.79±2.19b | 6.91±2.13b  | 5.21±1.76a  | 8.65±2.19c  | 0.65±0.01b  |
|                   | 0.06                              | 53.22±3.26a | 8.99±2.65a  | 5.66±3.13a  | 10.62±2.16a | 0.56±0.02c  |
| CK                |                                   | 46.61±5.25c | 6.48±1.02b  | 5.65±2.09a  | 8.60±2.05c  | 0.72±0.02a  |

3.3. **Internal fruit quality**

The Figure 1 revealed the effect on external fruit quality by spraying different concentration of brassinolide. The total soluble solid content revealed an increase trend with the increasing concentration of brassinolide, which were all significantly higher than that in the control group. The maximum TSS content reached 10.50% for 'Huangjinmiyou' and 9.80% for 'Hongroumiyou'. Spraying brassinolide significantly decreased the content of total acid, with the minimum value of 0.70% and 0.72% by 0.06 mg•L⁻¹ of brassinolide. Thus, spraying brassinolide effectively increased the TSS-acid ratio, which improved the taste quality of pummelo cultivars. Compared with the control group, vitamin C content showed a decrease change by spraying 0.02 and 0.04 mg•L⁻¹ of brassinolide, while it had a significant increase by 0.06 mg•L⁻¹ of brassinolide, reaching 28.0 and 26.0 mg•100mL⁻¹. Similarly, total sugar content accumulated to 8.50 g•100mL⁻¹ for 'Huangjinmiyou' and 7.50 g•100mL⁻¹ for 'Hongroumiyou'. As a result, spraying 0.06 mg•L⁻¹ of brassinolide could effectively improve the internal fruit quality of 'Huangjinmiyou' and 'Hongroumiyou'.

4. **Discussion**

In this study, we analyzed the effect of spraying brassinolide on fruit quality of two pummelo cultivars. Spraying brassinolide could promote the longitudinal and transverse diameter, while had no obvious effect on the fruit shape index. Brassinolide could significantly decrease the peel thickness of 'Hongroumiyou', while revealed no obvious effect on 'Huangjinmiyou'. Previous study suggested that brassinolide promoted fruit quantity by increasing fruit setting rate and weight per fruit [11]. Here, weight per fruit and edible rate of 'Huangjinmiyou' and 'Hongroumiyou' revealed an increase trend with the increasing concentration of brassinolide. Granulation index is related to the fruit over-ripeness and lignification of cell wall [16]. Spraying brassinolide could ease granulation of segments, reaching the best effect of 0.06 mg•L⁻¹ brassinolide. This indicated that brassinolide might slow down the fruit maturing and lignification of cell wall.

Fruit coloration has become an important indicator of fruit commodity in the market [12]. Spraying brassinolide improved the flesh chromatism with orange-yellow fruit of 'Huangjinmiyou', and light red flesh of 'Hongroumiyou'. As for the internal fruit quality, spraying brassinolide could significantly increase the accumulation of total soluble solid, whereas decrease total acid content. This was consistent with the previous study [11]. The TSS-acid ratio continuously increased with the increase of brassinolide, obviously improving the taste quality, which reached more than 10. Vitamin C revealed a decrease - increase trend with the increasing concentration of brassinolide, reaching the maximum value at 0.06 mg•L⁻¹ of brassinolide. In addition, brassinolide could promote the accumulation of total sugar.

Overall, spraying 0.06 mg•L⁻¹ of brassinolide could effectively decrease the peel thickness, promote the flesh weight and edible rate, and ease granulation index, as well as significantly improve the content of total soluble solid, vitamin C and total sugar. This will provide related basis for high-quality and high-efficient production of pummelo cultivars in Sichuan Province.
Figure 1. Effect of spraying brassinolide on internal fruit quality of *Citrus grandis* cv. 'Huangjinmiyou' and 'Hongroumiyou'

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