Effects of different plant growth regulators on blueberry fruit quality

X. C. Zhang, Y. Q. Zhu, Y. N. Wang, C. Luo, X. Wang*
Institute of Pomology & Olericulture, Sichuan Agricultural University, Chengdu, Sichuan, China
*Corresponding Author

Abstract: In order to understand the effects of different plant growth regulators (PGRs) on blueberry fruit growth, various concentrations of Abscisic acid (ABA), Methyl jasmonate (MJ), Brassinolide (BR), Melatonin (MT) were sprayed on blueberry cv. ‘Brigita’ fruits. The results showed that all the PGRs put into effect on improving the quality of blueberry fruit. Comparing with the control plants no PGR spraying, 300 mg/L of MT treatment promoted effectively accumulation of the soluble sugar. ABA 20mg/L treatment increased effectively accumulation of anthocyanin, and significantly decreased titratable acid content. The treatment of MJ 10mg/L improved significantly the soluble solid content. The effect of the four PGRs treatments on appearance did not show obvious difference.

1 Introduction
Blueberry (Vaccinium Spp.) is a kind of perennial deciduous or evergreen shrubs fruit trees. It belongs to family Vacciniaceae, genus Vaccinium [8]. Blueberry is one of the rare blue plants on the earth [11]. It has tiny seeds, soft pulp with special flavor, moderate sweet and sour taste [10]. The anthocyanin content of blueberry is far more than other fruits or vegetables. Anthocyanin has high value for health care. It possess strong oxidation resistance and anti-cancer function, which is able to soften blood vessels, strengthen the heart, improve the vision, relieve eye fatigue, prevent brain aging and enhance immunity. Blueberry is also rich in vitamin E, vitamin A, vitamin B, superoxide dismutase (SOD), as well as some trace elements like K, Z, Fe and Mn. British nutritionists name it as the first in 15 kinds of health food. Blueberry is also one of the five healthy foods acknowledged by United Nations FAO, and it is called "the king fruit" [13].

Exogenous plant growth regulators (PGRs) are influential in the internal and external quality of fruit. Abscisic acid (ABA) plays a positive role not only in promoting plant dormancy, improving stress resistance and information transmission, but also in enhancing the accumulation of photosynthetic products and increasing pigment content. The successful application of ABA have already been reported in grape, apple, sweet cherry and peach [6, 7, 16, 17].Methyl jasmonate (MJ) is a natural compound extracted from vegetable oil, and was confirmed as a harmless matter to human body by U.S. FDA [24]. MJs made fruit mature earlier, release the chilling injury, as well as improving the resistance to disease. It was reported that MJs help to increase the ability of antioxidant in postharvest fruits. MJ treatment enhanced the activities of antioxidants and antioxidant enzymes (including SOD, POX, APX, MDHAR, DHAR, etc.) in fruit [1]. In addition, MJ treatment pre-harvest was able to increase the contents of anthocyanin and total phenol significantly in fruits [21]. Brassinolide (BR) is a steroidal compounds extracted from rape pollen [5]. It is considered as the sixth plant endogenous
hormones following the five before (auxin, gibberellin, cytokinin, abscisic acid and ethylene). Application of BR can enhance soluble solids and sugar content, and reduce titratable acid content of fruit (grape, citrus and strawberry) in varying degrees, which is favor of sugar acid ratio and fruit taste improvement [9]. Melatonin (MT) is a kind of endogenous indole amine in all plants [15], and is detected in trace amounts in animals, and microbes [3]. MT is a kind of healthy ingredient for human body. Many fruits, including apple, cherry, banana, strawberry, pineapple, grape and tomato can be the source of MTs [14, 19]. Exogenous MT treatment was proved to put tomato fruits ripening ahead and improve fruit quality [20].

In this experiment, four kinds of PGRs were used to treat blueberry fruits. We studied the influence of PGRs on the main internal and appearance quality of fruit to make clear the application effect of different PGRs in blueberry production. This research is expected to be great practical significance for blueberry production and improve fruit quality, and to provide theoretical basis for application in the cultivation of blueberry.

2 Materials & Methods

2.1 Plant materials
Blueberry cv. 'Brigita' was used. The plants are growing in Sichuan Qionglai, NanBao mountain blueberry orchard. Their growth status was basically the same.

2.2 Experimental design
PGRs spraying was conducted twice, in 11th May and 8th June 2016. The concentrations of PGRs were listed in table 1, and water spraying was the experiment control. Each treatment was repeated for 3 times.

Table 1 The different concentrations of PGRs

| PGR | Concentration (mg/L) |
|-----|----------------------|
| ABA | 5  10  20            |
| MJ  | 5  10  20            |
| BR  | 0.1 0.3 0.6         |
| MT  | 100 200 300         |

* One kind of PGR treatment contains three levels of concentrations.

2.3 Fruit appearance quality estimation
Fruits were harvested on 20th July 2016. The fruits were measured in weight, vertical diameter and horizontal diameter to estimate the appearance quality. The weight were determined by randomly selecting 50 fruits per treatment, wiping off the surface moisture and residues, and weighing it with an electronic balance. The vertical and horizontal diameters were determined by randomly selecting 20 fruits per treatment, measuring it with vernier caliper.

2.4 Fruit internal quality examination
Fruits soluble solids content, soluble sugar content, titratable acid content and anthocyanin content were examined. Soluble solids content were measured with a hand-held glucose meter. Soluble sugars content were measured by anthrone colorimetry [22]. Standard titrimetric method for the determination of titratable acids content. Anthocyanin content were determined by Spectrophotometry [22].

2.5 Statistical method
Statistical analysis of experimental data was performed using Excel2007 and SPSS18.0, and the single factor analysis of variance was performed using Duncan's new bipolar difference method.
3 Results

3.1 Effects of different PGRs on fruits appearance quality

The effects of different PGRs and control treatment on single fruit weight were not significantly different \( (p > 0.05) \). Fruit weight treated with ABA 10mg/L was the lowest (1.433g), and fruit weight treated with MT 300mg/L was the highest (1.797g). The difference between fruits lowest and highest weights was significant \( (p < 0.05) \). ABA 10mg/L treatment and MJ 5mg/L treatment reduced the fruit weight. Under treatments of MJ (5mg/L, 10mg/L, 20mg/L), BR (0.1mg/L, 0.3mg/L, 0.6mg/L) and MT (100mg/L, 200mg/L, 300mg/L), the single fruit weight increased along with the raise of PGRs concentration.

The effects of different PGRs on the longitudinal and transverse diameter of blueberry were not significantly different \( (p > 0.05) \). With the raise of MT concentration, the longitudinal diameter of blueberry showed a trend decreasing, and the transverse diameter showed a trend increasing.

There was no significant difference between PGRs treatments and control on the fruit shape index \( (p > 0.05) \). Along with the raise of ABA and MT concentration, the fruit shape index showed a trend decreasing. The index of ABA 5mg/L treatment (0.833) was significantly higher than that of MJ 5mg/L treatment (0.748) and MT 300mg/L treatment (0.730) \( (p < 0.05) \).

3.2 Effects of different PGRs on fruits sugar and titratable acid content

The effects of different PGRs on soluble sugar and titratable acid content of blueberry fruits were different from those of control. In the treatments of ABA 10mg/L, ABA 20mg/L, MJ 10mg/L, MT 300mg/L and all concentration of BR, the soluble sugar contents were significantly improved compared with the control \( (p < 0.05) \). Along with the increase of ABA, BR and MT concentrations, the soluble sugar content of blueberry fruit increased. However, it increased first and then decreased with the raise of MJ concentration. Sorting of all treatments showed that, MT 300mg/L (9.56g/100g)>ABA 10mg/L (9.50g/100g)>ABA 20mg/L (9.12g/100g)>BR 0.6mg/L (8.56g/100g)>MJ 10mg/L (7.83g/100g). The titratable acid content of ABA 20mg/L treatment was significantly lower than that of control \( (p < 0.05) \). In contract, BR 0.3mg/L treatment, MT 200mg/L and 300mg/L treatments increased titratable acid contents significantly compared with the control \( (p < 0.05) \). With the raise of ABA and MJ concentrations, the contents of titratable acid decreased, but it showed a contrary trend with the increase of MT concentration. Sorting of all treatments showed that, ABA 20mg/L treatment (0.411g/100g)<ABA 10mg/L treatment (0.451g/100g)<MT 300mg/L treatment (0.459g/100g)<ABA 20mg/L treatment (0.501g/100g).

Table 2 The effect of different PGRs on the appearance quality of Blueberry

| Treatment | Single fruit weight(g) | Longitudinal diameter(cm) | Transverse diameter(cm) | Fruit shape index |
|-----------|------------------------|---------------------------|-------------------------|------------------|
| CK        | 1.509±0.054ab          | 1.229±0.047a              | 1.549±0.024ab           | 0.795±0.023ab    |
| ABA-5     | 1.560±0.058ab          | 1.134±0.053a              | 1.373±0.038b            | 0.833±0.054a     |
| ABA-10    | 1.433±0.200b           | 1.201±0.050a              | 1.520±0.026ab           | 0.790±0.002ab    |
| ABA-20    | 1.553±0.136ab          | 1.122±0.018a              | 1.476±0.037ab           | 0.762±0.002ab    |
| MJ-5      | 1.488±0.005ab          | 1.096±0.040a              | 1.469±0.015ab           | 0.748±0.014b     |
| MJ-10     | 1.620±0.004ab          | 1.163±0.022a              | 1.508±0.042ab           | 0.772±0.031ab    |
| MJ-20     | 1.645±0.065ab          | 1.170±0.042a              | 1.546±0.038a            | 0.757±0.013ab    |
| BR-0.1    | 1.582±0.036ab          | 1.161±0.043a              | 1.486±0.128b            | 0.783±0.023ab    |
| BR-0.3    | 1.781±0.203ab          | 1.139±0.043a              | 1.516±0.027ab           | 0.751±0.017ab    |
| BR-0.6    | 1.699±0.005ab          | 1.117±0.043a              | 1.476±0.027ab           | 0.759±0.028ab    |
| MT-100    | 1.629±0.105ab          | 1.196±0.035a              | 1.525±0.022ab           | 0.785±0.001ab    |
| MT-200    | 1.733±0.022ab          | 1.183±0.046a              | 1.545±0.060ab           | 0.766±0.006ab    |
| MT-300    | 1.797±0.111a           | 1.125±0.015a              | 1.554±0.026ab           | 0.730±0.040b    |
CK is the experiment control. The lower case letters have a significant difference in the level of 0.05. Similarly hereinafter.

Table 3 The effects of different PGRs on the inner quality of Blueberry

| Treatment | Soluble sugar (g/100g) | Titratable acid (g/100g) |
|-----------|------------------------|--------------------------|
| CK        | 5.67±0.390de           | 0.536±0.010cde           |
| ABA—5     | 6.85±0.444cde          | 0.501±0.007cde           |
| ABA—10    | 9.50±0.904a            | 0.451±0.062ef            |
| ABA—20    | 9.12±1.047ab           | 0.411±0.068f             |
| MJ—5      | 5.57±0.414de           | 0.578±0.007bc            |
| MJ—10     | 7.83±0.186abc          | 0.536±0.008cde           |
| MJ—20     | 5.43±0.330e            | 0.492±0.052cdef          |
| BR—0.1    | 7.72±0.402abc          | 0.563±0.018bcd           |
| BR—0.3    | 7.75±0.763abc          | 0.661±0.026b             |
| BR—0.6    | 8.56±0.273abc          | 0.549±0.053cde           |
| MT—100    | 5.25±0.047e            | 0.459±0.049def           |
| MT—200    | 7.35±0.473bcd          | 0.814±0.009a             |
| MT—300    | 9.56±0.575a            | 0.846±0.015a             |

3.3 Effects of different PGRs on fruits soluble solids and anthocyanins contents

The effects of different PGRs on the soluble solids and anthocyanins content of blueberry were different from those of control. Soluble solids contents of ABA treatments, MJ treatments, BR 0.3mg/L and 0.6mg/L treatment were significantly higher than those of control ($p<0.05$). With MJ and BR concentrations raised, the contents of soluble solids increased first and then decreased. The effect of MT treatment on blueberry was not obvious. Sorting of all treatments showed that, MJ 10mg/L (11.1%) > ABA 5mg/L (10.9%), BR 0.3mg/L (10.9%) > ABA 20mg/L (10.8%) > MJ 5mg/L (10.6%).

The anthocyanin content of blueberry treated with 10mg/L ABA was significantly higher than that of control ($p<0.05$). With the increase of ABA and MJ concentrations, the contents of anthocyanin in blueberry increased. Contrarily, the contents of anthocyanin dropped when BR concentration increased. In MT treatments, anthocyanin content increased following decreased.

Table 4 The effects of different PGRs on the inner quality of Blueberry

| Treatment | Soluble solids (%) | Anthocyanin (nmol/g) |
|-----------|--------------------|----------------------|
| CK        | 9.6±0.173fg        | 1184.9±43.255bcde    |
| ABA—5     | 10.9±0.133ab       | 1344.2±59.800abcd    |
| ABA—10    | 10.1±0.176cde      | 1461.9±122.22ab      |
| ABA—20    | 10.8±0.058ab       | 1596.3±177.585a      |
| MJ—5      | 10.6±0.153abc      | 923.5±84.820de       |
| MJ—10     | 11.1±0.265a        | 1129.8±130.425cde    |
| MJ—20     | 10.4±0.186bcd      | 1418.8±218.590abc    |
| BR—0.1    | 10.1±0.145def      | 1424.9±90.995abc     |
| BR—0.3    | 10.9±0.033ab       | 1393.8±63.050abc     |
| BR—0.6    | 10.3±0.153cde      | 1043.2±92.330de      |
| MT—100    | 9.2±0.176g         | 1047.2±7.910de       |
| MT—200    | 9.8±0.115ef        | 1373.7±36.865abc     |
| MT—300    | 9.8±0.145ef        | 1150.7±52.530cde     |
4 Discussions
Single fruit weight and fruit shape index reveal fruit appearance quality, and single fruit weight also represents the yield of blueberry. The contents of soluble sugar, titratable acid and soluble solids were important indicators of fruit internal quality, which reflect the fruit taste and flavor. As a kind of water soluble pigment of plant, anthocyanin is the most effective antioxidant bioactive agent for human’s healthy. In view of the special effects of anthocyanin, blueberry anthocyanin content will affect its economic value and nutritional value.

PGRs widely involve in the growth and development of plant, which produce effects on fruit quality in many aspects. They can directly enlarge the fruit by promoting cell growth and division, or indirectly affect the primary and secondary metabolites accumulation by participating in the defense of adversity stress. In this research, the effect of ABA on the appearance quality of blueberry fruit was not significant. Spraying ABA on blueberry raised the content of soluble sugar, soluble solids and anthocyanin, and reduced the content of titratable acid. The results were consistent with the conclusions of Yu et al. [23] on ‘Jingyou’ grape and Sun et al. [18] on blueberry. The effect of MJ on the appearance quality of blueberry fruit did not showed significant difference as well. Appropriate concentration of MJ enhanced the contents of soluble sugar, soluble solids and anthocyanin, but the content of titratable acid decreased. Along with the increase of BR spraying concentration, single fruit weight increased and fruit shape index decreased, but no significant difference from the experiment control. Comparing with the researches of Feng et al. [4] on 'Red Globe' grape and Ma et al. [12] on Cabernet Gernischt, the results in this research are same on single fruit weight but opposite on fruit shape index. Differences on fruit shape index may be due to the plant species inherent characteristics. Spraying BR on blueberry raised the content of soluble sugar, soluble solids and anthocyanin, but did not affect titratable acid content significantly. The results were consistent with the conclusions on strawberry, cucumber, grape, citrus [9] and Ma et al. [12] on Cabernet Cabernet. The effect of spraying MT on the appearance quality of blueberry fruit was similar to the influence from spraying BR in our research. Spraying MT raised the content of soluble sugar on blueberry, which was consistent with the results of Sun et al. on tomato [20]. However, spraying MT had not put obvious effect on the contents of soluble solids and anthocyanin.

5 Conclusions
To summarize, all PGRs treatments in this research did not display obvious effect to fruit appearance quality, but would enhance the quality of blueberry fruit in varying degrees. The MT 300mg/L treatment effectively promoted the accumulation of soluble sugar, in which treatment, the soluble sugar content increased by 68.6% comparing with the control fruits. The ABA 20mg/L treatment significantly reduced the content of titratable acid, and effectively promoted the accumulation of anthocyanins in blueberry fruit. The ABA 20mg/L treatment reduced the titratable acid content by 23.3% and improved the anthocyanin content by 34.8% in fruit comparing with the control. The MJ 10mg/L treatment significantly improved the content of soluble solids in blueberry fruit, which increased the soluble solids content by 15.6% comparing with the control.

Proper and selective spraying of these PGRs can enhance the economic value of blueberry fruits. We suggest that 300mg/L MT, 20mg/L ABA and 10mg/L MJ are all available for use on blueberry in actual production.

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