

## Effects of Shading on Carbohydrates of *Syzygium samarangense*

Ying JIAN<sup>1,a</sup>, Guolin WU<sup>2,b</sup>, Donghui ZHOU<sup>2,c</sup>, Zhuqun HU<sup>1</sup>,  
Zhenxuan QUAN<sup>1</sup>, Biyan ZHOU<sup>1\*</sup>

<sup>1</sup>South China Agricultural University, College of Horticulture, Guangzhou 510642, China; [491257864@qq.com](mailto:491257864@qq.com); [zhqhu@scau.edu.cn](mailto:zhqhu@scau.edu.cn); [185191743@qq.com](mailto:185191743@qq.com); [zboubiyan@scau.edu.cn](mailto:zboubiyan@scau.edu.cn) (\*corresponding author)

<sup>2</sup>Zhuhai Modern Agricultural and Developmental Center, Zhuhai 519070, China; [1293831688@qq.com](mailto:1293831688@qq.com); [910441075@qq.com](mailto:910441075@qq.com)

<sup>a,b,c</sup>These authors contributed equally to this work

### Abstract

Wax apple (*Syzygium samarangense*) is an important tropical fruit tree cultivated in Southeast Asian. It produces red pear-like shape fruits. The fruit flesh is considered high in antioxidants, phenolics, and flavonoids that have a potential to contribute to the human healthy diet, and was proved to have anti-inflammatory and antimicrobial characteristics. To allow year-round marketing of high quality wax apple fruit, growers always perform shading to inhibit new flushes so as to repress vegetative growth and promote reproductive growth. To investigate the effect of shading on carbohydrates, wax apple trees were shaded with sun shade nets under field conditions. The effects of shading on shoot growth were studied and leaf carbohydrate levels of the trees were determined. The results showed that shading inhibit the the growth of the terminal shoots and promoted bud dormancy. Shading also reduced total soluble sugar, sucrose, glucose, fructose, and starch levels of leaves. The results suggested that shading reduced carbohydrate accumulation and repressed vegetative growth.

**Keywords:** carbohydrates; shading; wax apple

### Introduction

Wax apple (*Syzygium samarangense*) is an important tropical fruit tree cultivated in Southeast Asian. It produces red pear-like shape fruits. The fruits are juicy, crisp, and refreshing. The fruit flesh is considered high in antioxidants, phenolics, and flavonoids that has a potential to contribute to the human healthy diet (Jamil *et al.*, 2018), and was proved to have anti-inflammatory and antimicrobial characteristics (Moneruzzaman *et al.*, 2012). Wax apple trees have nonseasonal flowers and can produce fruits timely under control. To allow year-round marketing of high quality wax apple fruits, growers always perform shading to inhibit new flushes so as to repress vegetative growth and promote reproductive growth (Cho *et al.*, 2018).

Shading can affect a wide range of physiological and biochemical processes. It is reported that shading reduced net photosynthetic rate, chlorophyll content and ribulose-1,5-bisphosphate carboxylase activity, as well as sucrose biosynthesis in cotton plants (Li *et al.*, 2019). However, how shading affects physiological and biochemical processes of wax apple trees, and the mechanism of shading on growth control in wax apple trees, still need further investigation.

In this study, wax apple trees were shaded with sun shade nets under field conditions. The effects of shading on shoot growth were studied and leaf carbohydrate levels of the trees were determined, with the aim to provide basic materials for controlling vegetative growth underlying flowering in wax apple trees.

### Materials and Methods

#### *Plant material and experiment procedures*

Eight-year-old wax apple (*Syzygium samarangense* cv. 'Heizhenzhu') trees were grown at Jinpu orchard in Zhongshan city. Trees were about 2.4 meters in height and with 200-250 terminal shoots. On June 26, 2015, when leaves of the terminal shoots turned green, 6 trees covered with 90% sun shade nets were as shading treatment. Another 6 trees with similar phenological stage without shading nets were as controls. Leaves of the terminal shoots (Shoot 1), the shoots growing before the terminal shoots (Shoot 2), and the shoots growing before shoot 2 (Shoot 3) were collected at 0, 11, 21, and 31 d of treatment. The third leaves of the shoots at four directions in one tree were sampled and pooled together, frozen in liquid nitrogen and stored at -80 °C for determination of starch content and soluble sugar content.

*Determination of soluble sugar and starch*

Samples were ground to powder and distilled water was added, incubated in a water bath at 90 °C for 30 min, and were centrifuged at 8 000 rpm for 15 min. The supernatant solution was used to determine soluble sugar, sucrose and fructose according to the method of Xue (1985), and glucose according to the method of Zhou (2004). Extraction and determination of starch are according to the method of Xu *et al.* (1998).

*Statistical analysis*

All data were subjected to analysis of variances using an SPSS program (SPSS Inc. Chicago, IL, USA). The differences between treatments and controls were evaluated by Student's *t*-test ( $P \leq 0.05$ ).

**Results***Shading inhibits growth of terminal shoots*

As shown in Fig. 1, new flushes were found in the control terminal shoots from 0 d to 31 d time points, whereas no flushes were found in the shading shoots. All the buds of the shaded shoots were still at the dormant stage, suggesting that shading repressed the growth of the terminal shoots and promoted bud dormancy.

*Effects of shading on total soluble sugar contents of leaves in different kinds of shoots*

As shown in Fig. 2, total soluble sugar contents of the shaded leaves in shoot 1, shoot 2, and shoot 3 increased from 0 d time point to 11 d time point then decreased, while those of the control leaves in shoot 1 and shoot 2

showed increasing trends. However, those of the control leaves in shoot 3 were relatively stable at the four day time points. On the whole, shading decreased the soluble sugar contents of leaves. For example, at 31 d time point, total soluble sugar contents of the shaded leaves in all the three type shoots were significantly lower than those of controls.

*Effects of shading on fructose contents of leaves in different kinds of shoots*

As shown in Fig. 3, fructose content of the shaded leaves in shoot 1 decreased to the lowest level at 21 d time point then increased to the control level at 31 d time point (Fig. 3 A), while that in shoot 2 and shoot 3 showed decreasing trends (Fig. 3 B, C). However, fructose contents of the control leaves in the three types of shoots were stable and remained at high levels. On the whole, shading decreased fructose contents. For example, at 11 d and 21 d time points, fructose contents of the shaded leaves in all the three types of shoots were significantly lower than those of controls.

*Effects of shading on sucrose contents of leaves in different kinds of shoots*

As shown in Fig. 4, sucrose contents of the shaded leaves in the three types of shoots showed similar trends, and decreased to the lowest level at 21 d time point then increased. Those contents of the control leaves in shoot 1 and shoot 2 increased from 0 to 31 d time points, whereas the content in shoot 3 showed relatively stable. On the whole, shading decreased sucrose levels of leaves. For example, at 21 d time point, sucrose contents of the shaded leaves in shoot 1, shoot 2, and shoot 3 were less than those of controls by 46.4%, 44.1%, and 33.2% respectively.

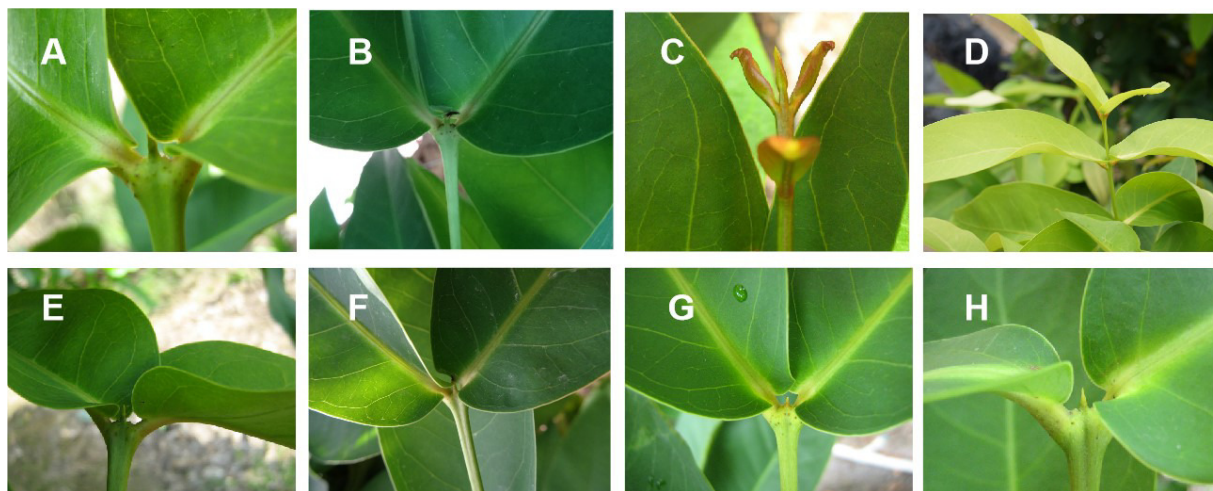


Fig. 1. Image of the terminal shoots showing the effects of shading on shoot growth from 0 d to 31 d of treatment. A-D, control shoots. E-F, shaded shoots. A, a control shoot at 0 d time point; B, a control shoot at 11 d time point; C, a control shoot at 21 d time point; D, a control shoot at 31 d time point; E, a shaded shoot at 0 d time point; F, a shaded shoot at 11 d time point; G, a shaded shoot at 21 d time point; H, a shaded shoot at 31 d time point.

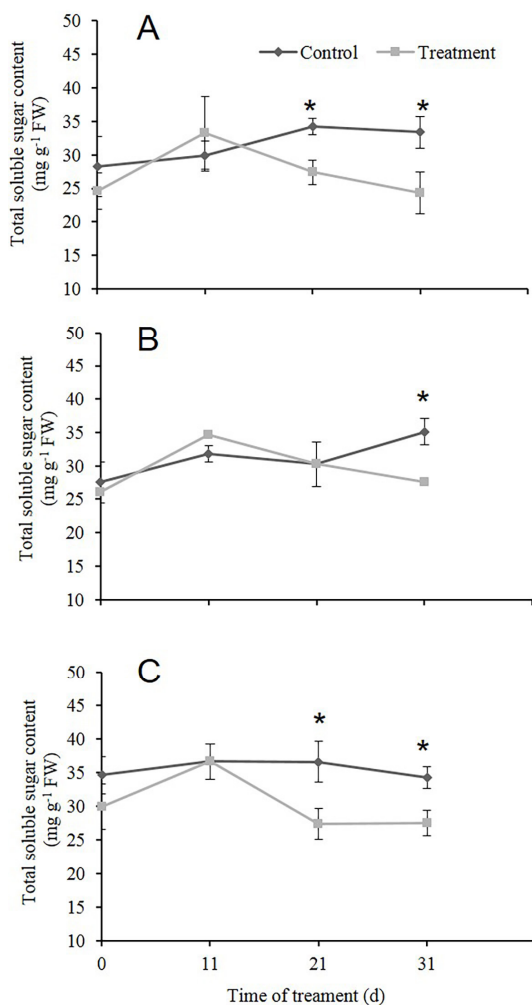


Fig. 2. Effects of shading on total soluble sugar contents of leaves in different kinds of shoots. Eight-year-old wax apple trees were covered with 90% sun shade nets as shading trees, and those without sun shade nets were as control trees. Leaves of the terminal shoots (shoot 1), the shoots growing before the terminal shoots (shoot 2), and the shoots growing before shoot 2 (shoot 3) were collected at 0, 11, 21, and 31 d of treatment. A, shoot 1; B, shoot 2; C, shoot 3. Asterisks indicate significant differences between treatments and controls evaluated by Student's *t*-test ( $P \leq 0.05$ ).

#### *Effects of shading on glucose contents of leaves in different kinds of shoots*

As shown in Fig. 5, glucose contents of the shaded leaves in shoot 1 and shoot 2 showed similar trends. They decreased to the lowest level at 21 d time point and then remained at a similar level at 31 d time point, while those of the control leaves in shoot 1 and shoot 2 were stable and remained at high levels (Fig. 5, A and B). However, the fructose content of the shaded and control ones in shoot 3 showed different trends (Fig. 5, C). On the whole, glucose contents of the shaded leaves in the three types of shoots were lower than those of the control leaves.

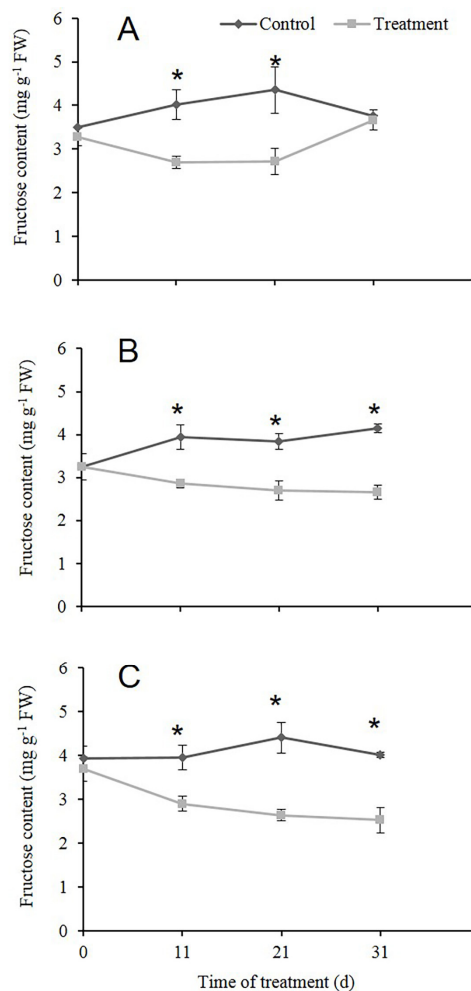


Fig. 3. Effects of shading on fructose contents of leaves in different kinds of shoots. Eight-year-old wax apple trees were covered with 90% sun shade nets as shading trees, and those without sun shade nets were as control trees. Leaves of the terminal shoots (shoot 1), the shoots growing before the terminal shoots (shoot 2), and the shoots growing before shoot 2 (shoot 3) were collected at 0, 11, 21, and 31 d of treatment. A, shoot 1; B, shoot 2; C, shoot 3. Asterisks indicate significant differences between treatments and controls evaluated by Student's *t*-test ( $P \leq 0.05$ ).

#### *Effects of shading on starch contents of leaves in different kinds of shoots*

As shown in Fig. 6, starch contents of the shaded leaves in the three types of shoots showed decreasing trends from 0 d time point to 31 d time, while those of the controls in shoot 1 and shoot 2 showed increasing trends. However, the starch content of the control leaves in shoot 3 was stable and as similar levels at all day time points. Shading significantly decreased the starch contents in the shoot 1 and shoot 2 at 11, 21, and 31 day time points, and significantly decreased those in shoot 3 at 11 and 21 d time points.

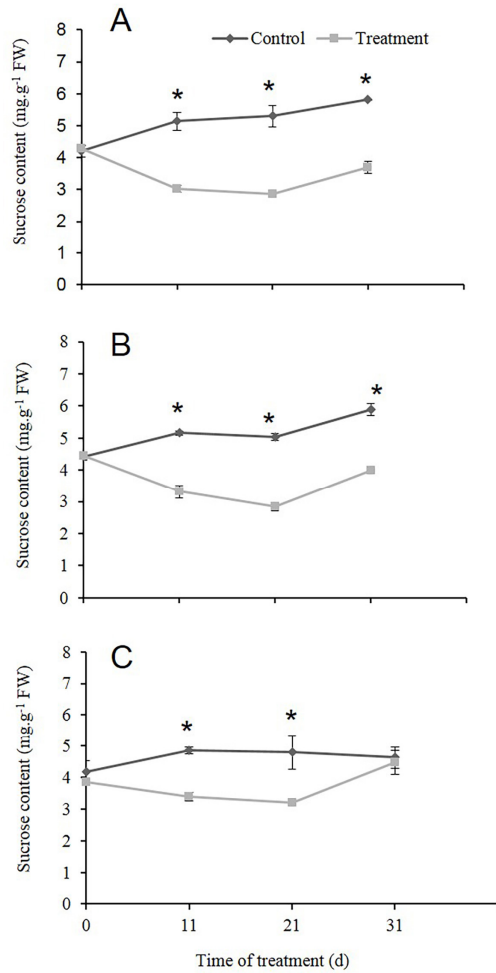


Fig. 4. Effects of shading on sucrose contents of leaves in different kinds of shoots. Eight-year-old wax apple trees were covered with 90% sun shade nets as shading trees, and those without sun shade nets were as control trees. Leaves of the terminal shoots (shoot 1), the shoots growing before the terminal shoots (shoot 2), and the shoots growing before shoot 2 (shoot 3) were collected at 0, 11, 21, and 31 d of treatment. A, shoot 1; B, shoot 2; C, shoot 3. Asterisks indicate significant differences between treatments and controls evaluated by Student's *t*-test ( $P \leq 0.05$ ).

**Discussion**

Carbohydrates include soluble sugar and starch. Sugars are resources for respiration and metabolic intermediates as well as structural components (Sheen *et al.*, 1999). Starch as an energy reservoir can be hydrolyzed to sugar. It has been emphasized that carbohydrates play an important role in flowering (Menzel and Simpson, 1988; Chen and Huang, 2005). Drought stress enhances carbohydrate accumulation and promoted flowering in *Averrhoa carambola* (Wu *et al.*, 2017). In the present study, we found that shading reduced total soluble sugar, sucrose, glucose, fructose, and starch levels of the leaves (Figs. 2-6), suggesting that shading reduced carbohydrate accumulation.

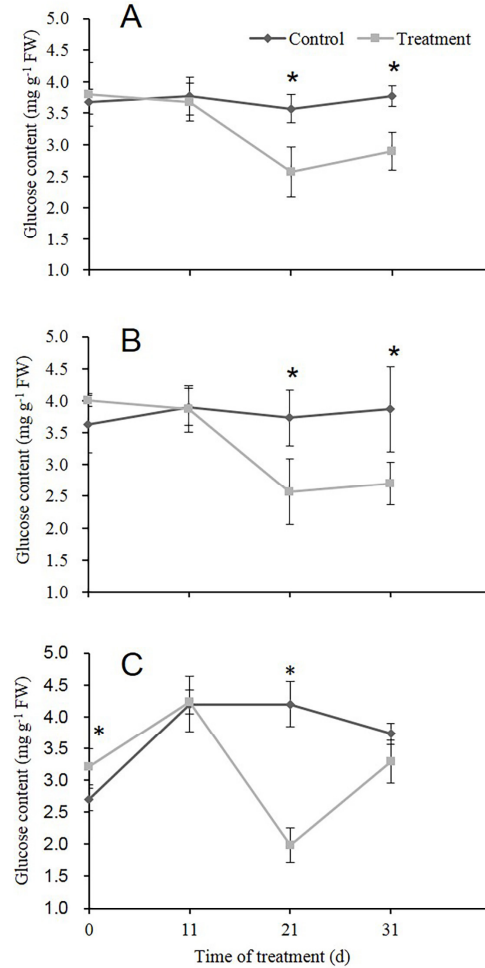


Fig. 5. Effects of shading on glucose contents of leaves in different kinds of shoots. Eight-year-old wax apple trees were covered with 90% sun shade nets as shading trees, and those without sun shade nets were as control trees. Leaves of the terminal shoots (shoot 1), the shoots growing before the terminal shoots (shoot 2), and the shoots growing before shoot 2 (shoot 3) were collected at 0, 11, 21, and 31 d of treatment. A, shoot 1; B, shoot 2; C, shoot 3. Asterisks indicate significant differences between treatments and controls evaluated by Student's *t*-test ( $P \leq 0.05$ ).

Many studies indicated that carbohydrate levels in shoots are positively correlated with flowering (Wu *et al.*, 2013; Yang *et al.*, 2014). According to the results of this study that shading reduced leaf carbohydrates, it should be that shading reduce flowering in wax apple. On the contrary, and quite different from that of *Medicago sativa* (Lorenzo *et al.*, 2019), shading can promote flowering in wax apple. Growers always use 90% or 95% sun shade nets for shading to repress new flushes and force buds to prolong dormant stage. In our previous study, we also found that shading promoted flowering in wax apple (Quan *et al.*, 2018).

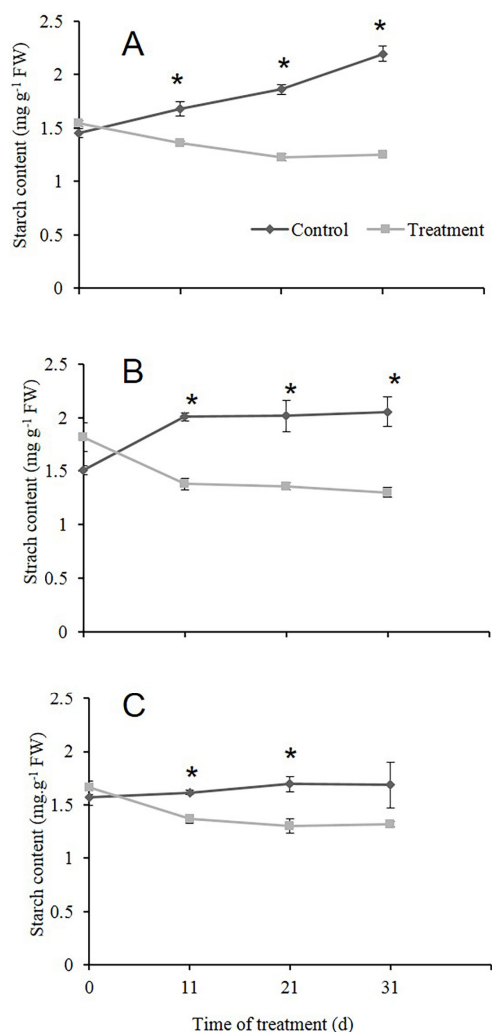


Fig. 6. Effects of shading on starch contents of leaves in different kinds of shoots. Eight-year-old wax apple trees were covered with 90% sun shade nets as shading trees, and those without sun shade nets were as control trees. Leaves of the terminal shoots (shoot 1), the shoots growing before the terminal shoots (shoot 2), and the shoots growing before shoot 2 (shoot 3) were collected at 0, 11, 21, and 31 d of treatment. A, shoot 1; B, shoot 2; C, shoot 3. Asterisks indicate significant differences between treatments and controls evaluated by Student's *t*-test ( $P \leq 0.05$ ).

It was suggested that a decrease in leaf carbohydrate levels by shading did not hamper flowering. Interestingly, we found that shading completely repressed new flushes and allowed bud dormancy (Fig. 1). In our previous study, the distribution of starch grains in cells of the apical buds was imaged by paraffin sections. To our surprise, quite different from leaves, we found that starch grain could be found before shading and they could be still seen in the cells during the shading treatment. However, in the control trees, they disappeared with the growing of the new flushes. It is suggested that shading might maintain a high concentration of starch in buds by repressing the growth of new flushes.

This may be the reason why shading can promote flowering. However, the mechanism of shading in relation to other kinds of carbohydrates, and in relation to the flowering related gene expression still calls for further study.

## Conclusions

Shading repressed the the growth of the terminal shoots and promoted bud dormancy. Shading also reduced total soluble sugar, sucrose, glucose, fructose, and starch levels of leaves.

## Acknowledgements

This study was supported by Guangdong Science and Technology Project, grant number 2015A020209127, and the provincial Natural Science Foundation, grant number 2017A03013161.

## Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

## References

- Chen HB, Huang HB (2005). Low temperature requirements for floral induction in lychee. *Acta Horticulturae* 665:195-202.
- Cho A, Chen N, Paull RE (2018). Modification of production systems for year-round marketing. *Acta Horticulturae* 1205:191-201.
- Jamil NAM, Rahmad N, Rosli NHM, Al-Obaidi JR (2018). Proteomic and metabolomic study of wax apple (*Syzygium samarangense*) fruit during ripening process. *Electrophoresis* 39(23):2954-2964.
- Li T, Dai JL, Zhang YJ, Kong XQ, Li CD, Dong HZ (2019). Topical shading substantially inhibits vegetative branching by altering leaf photosynthesis and hormone contents of cotton plants. *Field Crop Research* 238:18-26.
- Lorenzo CD, Iserte JA, Lamas MS, Antonietti MS, Gagliardi PG, Hemando CE, ... Yanovsky MJ (2019). Shade delays flowering in *Medicago sativa*. *Plant Journal* 99(1):7-22.
- Menzel CM, Simpson DR (1988). Effect of temperature on growth and flowering of litchi (*Litchi chinensis* Sonn.) cultivars. *Journal of Horticultural Science* 63(2):349-360.
- Moneruzzaman KM, Nasrulhaq Boyce A, Osman N, Sharif Hossain ABM (2012). Physiochemical and phytochemical properties of wax apple (*Syzygium samarangense* [Blume] Merrill & LM Perry var. 'Jambu Madu') as affected by growth regulator application. *The Scientific World Journal* 728613:1-13.
- Quan Z, Wei D, Wu G, Zhou D, Huang X, Zhou B (2018). Changes of structure of apical buds and distribution of starch grains in wax apple during flowering formation. *Journal of Fruit Science* 35(7):845-852 (in Chinese).
- Sheen J, Zhou L, Jang JC (1999). Sugars as signaling molecules. *Current Opinion in Plant Biology* 2(5):410-418.
- Wu P, Zhou B, Chen J (2013). Changes in carbohydrate in branches and its relation to flowering in *Averrhoa carambola*. *Acta Horticulturae*

- 975:433-439.
- Wu P, Wu C, Zhou B (2017). Drought stress induces flowering and enhances carbohydrate accumulation in *Averrhoa Carambola*. Horticultural Plant Journal 3(2):60-66.
- Xu CJ, Chen WJ, Chen KS, Zhang SL (1998). A simple method for determining the content of starch-iodine colorimetry. Biotechnology 8:41-43 (in Chinese).
- Xue YL (1985). Plant physiology manual. Shanghai Science and Technology Publication, Shanghai pp 134-138.
- Yang H-F, Kim H-J, Chen H-B, Rahman J, Lu X-Y, Zhou B-Y (2014). Carbohydrate accumulation and flowering-related gene expression levels at different developmental stages of terminal shoots in *Litchi chinensis*. HortScience 49(11):1381-1391.
- Zhou BY (2004). Responses of *Stylosanthes guianensis* to chilling and its regulation by abscisic acid. PhD Thesis, South China Agricultural University, Guangzhou (in Chinese).