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Research Article

# Effects of shading on photosynthetic characteristics of wax apple leaves

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## Abstract

The wax apple (*Syzygium samarangense*) is a highly valuable fruit species in Southeast Asia. To regulate the fruiting season, shading is commonly used to induce flowering in wax apple. However, the effects of shading on the growth of wax apple is not well understood. To address this, we conducted a study analyzing the photosynthetic characteristics of wax apple leaves under 40% and 90% shading rates. Our findings revealed that shading had a significant impact on the photosynthesis and branching tip development of wax apple. During shading treatments, the chlorophyll contents of the leaves increased to enhance light absorption efficiency. In the 40% shading treatment, the primary factor causing the decrease in net photosynthetic rate was stomatal limitation, while in the 90% shading treatment, both stomatal and non-stomatal limitations contributed to the decrease in net photosynthetic rate. These results are indications that sheading plays a key role in chlorophyll and photosynthesis in wax apple. These results will have led to a new research direction for genetic crop improvement.

***Keywords:*** chlorophyll contents; flowering; photosynthetic characteristics; shading; wax apple

## Introduction

The wax apple, also known as the Java apple, is a type of evergreen fruit tree that belongs to the Myrtaceae family. It is a highly valued cash crop in Southeast Asia and has earned the nickname "fruit emperor" due to its popularity (Shü *et al*., 2008). Thanks to the guidance of Taiwanese entrepreneurs, the cultivation area of wax apple in mainland China has been expanding and has brought significant economic benefits (Zheng *et al*., 2016). However, typically this fruit has a high production only in July and August, which often leads to market saturation and a decrease in price from RMB 50 to RMB 20 (Xu *et al*., 2020). Moreover, the high temperatures and humidity during this time make it difficult to control pests and diseases, which can negatively impact the yield and quality of the fruit (Weng *et al*., 2021).

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The method of controlling the fruiting season of wax apple is widely used in agriculture. In environments with insufficient light, plants tend to grow excessively without producing flowers. However, shading can actually promote flowering in wax apple (Yang, 2004). Therefore, shading is a crucial technique for regulating the fruiting season. By using shading, the tree's buds are forced into dormancy, preventing the consumption of nutrients by new shoots and improving the rate of flowering branches. During the peak summer season, the growth of wax apple is very vigorous. Therefore, black netting can be used to control the length of the shoots, and cultivation measures such as girdling, root pruning, and soaking can be employed to regulate its nutritional growth and achieve the goal of adjusting the tree's vigor (Cho *et al*., 2018). Additionally, effective chemical agents can be used to promote the emergence of flower buds and advance the fruiting season to around the time of the Spring Festival, thereby enhancing competitiveness in the market (Chang *et al*., 2000).

The process of photosynthesis is crucial for plants as it converts solar energy into a form that can be utilized. This process not only provides energy for the plant but is also vital for the development of almost all life on Earth. Additionally, light serves as a signal that influences the growth and development of plants (Ezer *et al*., 2017). To adapt to their surroundings, plants modify their morphology, physiology, and ecological characteristics in response to varying light conditions (Yang *et al*., 2014). When exposed to shade, plants typically experience a reduction in their net photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr), but an increase in their light compensation point (LCP), stomatal limitation value (Ls), and water use efficiency (WUE). The physiological responses of higher plants to light intensity differ due to individual differences, resulting in adaptive differences in the distribution ratios of absorbed light energy, photochemical reactions, thermal dissipation, and cyclic electron transfer capacity under different light intensities.

Replace with the Wax apple is a main economic fruit specie in Southeast Asia. However, it is currently facing challenges related to fruit quality, pests, and diseases, which are negatively impacting its yield and quality. To address this issue, local farmers must regulate the fruiting season to maximize their economic profits. This study aims to investigate the impact of shading on the nutritional growth and photosynthetic characteristics of 'Yinduhong' wax apple. Additionally, it explores the photosynthetic characteristics of wax apple during shading to facilitate the development of wax apple production and provide a theoretical basis for improving off-season cultivation techniques.

## Materials and Methods

### *Plant material*

The source of the materials used in this study were 4-year-old 'Yinduhong' wax apple trees that were cultivated at the orchard of the College of Horticulture, South China Agricultural University, located in Guangzhou, Guangdong Province (113° 21′ E, 23° 9′ N). The trees were planted with a spacing of 2 m * 2 m, and had an average height and canopy width of approximately 2 m. Each tree had between 40 to 50 terminal branches.

### *Sample collection process*

Starting on September 20th, 2019, a regular sampling schedule was implemented, with samples being collected every six days between the hours of 8:00 am and 10:00 am. The collection site was the mature leaves located at the end of branches, with only the middle section being retained after trimming the base and tip. To preserve the integrity of the samples, the leaves were flash-frozen in liquid nitrogen immediately after collection and stored in an ultra-low temperature freezer at -80 °C.

*A statistical survey on horticultural traits*

On September 20, 2019, an experiment was conducted to study the effects of shading on the growth of 'Yinduhong' wax apple trees. A total of twelve uniform trees were selected and divided into three groups, each consisting of four biological replicates. The shading treatments were set at 40%, 90%, and 0% (control) shade intensity. The shading determination criteria based on photosynthetically active radiation external (PARe) are 0% (1448.857 ± 41.271 $\mu molm^{-2}s^{-1}$), 40% (575.667 ± 14.012 $\mu molm^{-2}s^{-1}$), and 90% (146 ± 23.033 $\mu molm^{-2}s^{-1}$). And the experiment lasted for 30 days.

To evaluate the impact of shading on the growth of the trees, the number of shoots and branch lengths were recorded at 0 d, 15 d, and 30 d for each group. Record the number and length of branches for each experimental group at 0 d, 15 d, and 30 d, and hang tags on them. Determine the number of new shoots at 15 d and 30 d based on the count of different colored tags. By subtracting at a 15 d interval, the branch growth rate is obtained. ANOVA test and Tukey's test statistical analyses were conducted to determine the significance of the results. Furthermore, the photosynthetic parameters of wax apple leaves were measured using a TARGAS-1 portable photosynthesis instrument (PP-System Brand) at 1 d, 3 d, 5 d, and 7 d after removing the shading nets. This was done to assess the recovery of the trees after being subjected to different levels of shading.

*Determination of parameters of photosynthesis, chlorophyll fluorescence and chlorophyll content*

To conduct the shading treatment, we carefully selected the last shoot on the east, south, west, and north of each group. We then identified and marked the mature and uniformly growing third leaf from the top. To monitor the photosynthetic parameters of each group's leaves, we measured them every 6 days (TARGAS-1 portable photosynthesis instrument). To further analyze the leaves, we collected fresh samples from the last shoot and wrapped them in moist cotton at the base. Using the chlorophyll fluorescence imaging system (IMAGINE PAM) in the public experimental laboratory of the College of Horticulture, we conducted measurements and according to the method described by Ren *et al*. (2012), the chlorophyll content is measured using a NanoDrop 2000 UV spectrophotometer (Thermo Fisher Brand).

## Results

*Effect of shade treatment on the growth of wax apple branch tips*

During early spring season, branching tip development are one of the key factors for plant growth and production. We investigate the effect of shading of wax apple on branching tip development. The results showed that during the 15 d and 30 d shading treatments, the T1 group (40% shading) and T2 group (90% shading) exhibited a decrease in the number of new shoots and branch growth compared to the control. Notably, the T2 group showed a significant difference in comparison to both the T1 group and control group throughout the entire study period (Figure 1).

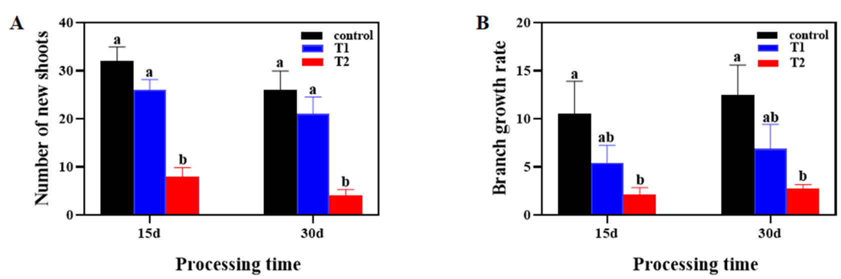


**Figure 1.** Effect of different levels of shade treatment on the number of new shoots **(A)** and branch growth rate **(B)** of wax apple. T1 group is 40% shading and T2 group is 90% shading. Bars with the same letters (a – b) were not significantly ($p < 0.05$) different after ANOVA test and Tukey's test

*Effect of shading treatment on photosynthetic parameters of wax apple leaves*

Throughout the shading process, a decline in photosynthetic rate (Pn), transpiration rate (Tr), and stomatal conductance (Gs) values were observed in both the T1 and T2 groups, while the control showed no significant change (Figure 2). The Pn, Tr, and Gs values of the T1 and T2 groups were notably lower than those of the control, and the difference was statistically significant. The intercellular $CO_2$ concentration (Ci) values of the T1 and T2 groups were also lower than those of the control. However, the T1 group exhibited a significant difference from the control group only at 18 d, whereas the T2 group showed significant differences at 12 d, 18 d, and 24 d. The T2 group had lower Pn and Tr values than the T1 group, and the difference between them was significant. The Ci and Gs values of the T2 group were also lower than those of the T1 group, but significant differences were only observed at 12 d, 18 d, and 30 d.

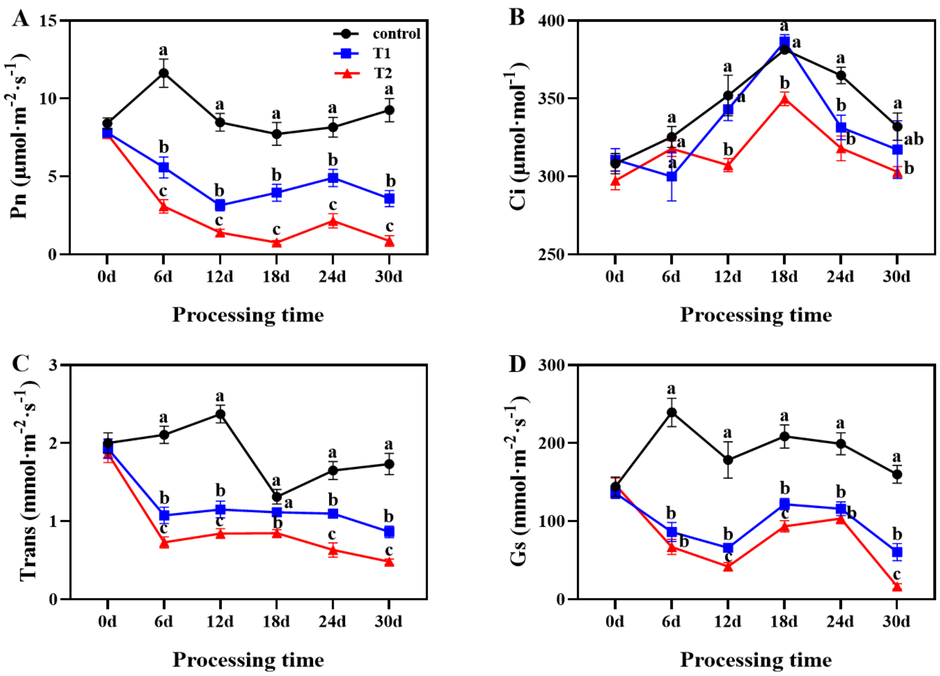


**Figure 2.** Effect of shading on photosynthetic parameters of wax apple leaves. **(A)** Changes in Pn values. **(B)** Changes in Ci values. **(C)** Changes in Tr values. **(D)** Changes in Gs values. T1 group is 40% shading and T2 group is 90% shading. Bars with the same letters (a – c) were not significantly ($p < 0.05$) different after ANOVA test and Tukey's test

*Changes in photosynthetic parameters of wax apple leaves after release shading*

After undergoing 30 d of shading treatment, both the T1 and T2 groups displayed an overall upward trend and were able to recover to the control level at approximately 7 d (Figure 3). Throughout the period of change, the Pn, Tr, and Gs values of the T1 and T2 groups were consistently lower than those of the control. However, the significant difference between the T1 group and control disappeared at 5 d, while the significant difference between the T2 group and control disappeared at 7 d. Similarly, the Ci values of the T1 and T2 groups were also lower than those of the control as a whole. The difference between the T1 group and the control group disappeared on the 3 d, while the significant difference between the T2 group and the control group persisted until the 7 d.

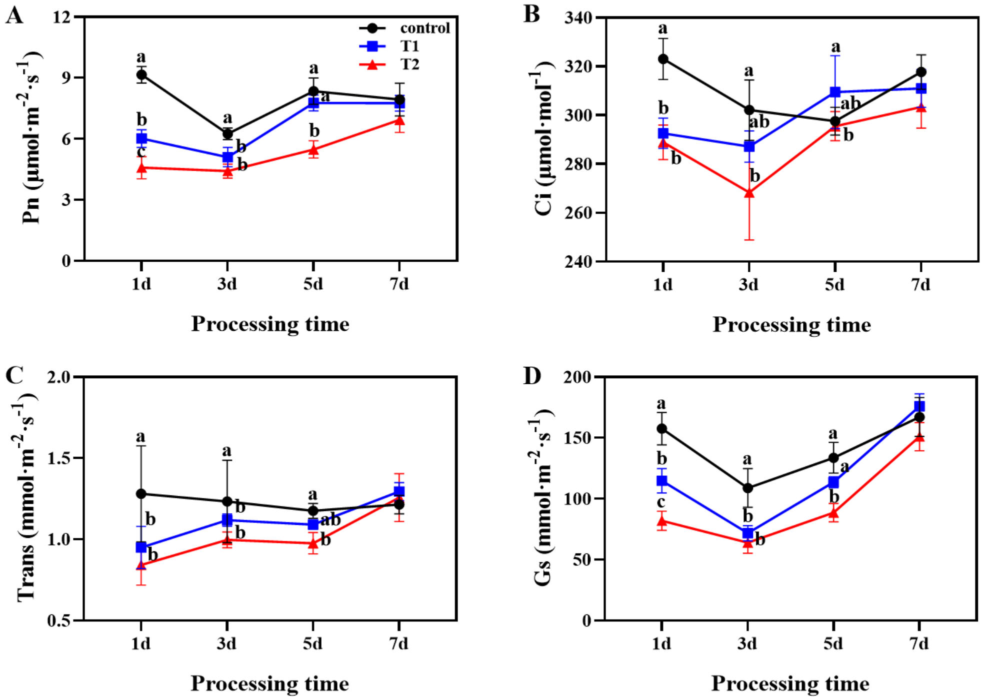


**Figure 3.** Effect of shading relief on photosynthesis-related parameters in wax apple leaves. **(A)** Changes in Pn values. **(B)** Changes in Ci values. **(C)** Changes in Tr values. **(D)** Changes in Gs values. T1 group is 40% shading and T2 group is 90% shading. Bars with the same letters (a – c) were not significantly ($p < 0.05$) different after ANOVA test and Tukey's test

*Effect of shading treatment on chlorophyll content of wax apple leaves*

During the shading process, the wax apple leaves exhibited an upward trend in chlorophyll a content, chlorophyll b and total chlorophyll contents over time. The T1 and T2 groups showed a greater increase compared to the control (Figure 4). At 12 d, the total chlorophyll content, chlorophyll a content, and chlorophyll b content of the T1 and T2 groups were significantly higher than those of the control. At 24 d, the total chlorophyll content of the T1 and T2 groups was significantly higher than that of the control. Although there was no significant difference in chlorophyll a content, the chlorophyll b content of the T1 group was significantly different from that of the control. At 30 d, the chlorophyll a content and chlorophyll b content of the T1 and T2 groups were significantly higher than those of the control. Only the total chlorophyll content of the T2 group was significantly different from that of the control.

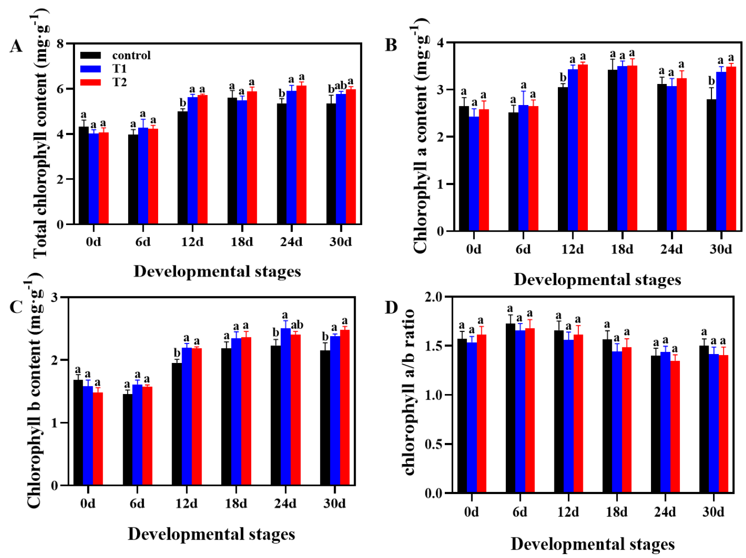


**Figure 4.** Effect of shading on chlorophyll content of wax apple leaves. **(A)** Changes in total chlorophyll content. **(B)** Changes in chlorophyll a content. **(C)** Changes in chlorophyll b content. **(D)** Changes in chlorophyll a/b ratio. T1 group is 40% shading and T2 group is 90% shading. Bars with the same letters (a – b) were not significantly ($p < 0.05$) different after ANOVA test and Tukey's test

*Effect of shading treatment on chlorophyll fluorescence parameters of wax apple leaves*

Throughout the 30-d shading period for wax apples, both the T1 and T2 groups experienced a decline in Fv/Fm, photosynthetic electron transfers rate (ETR), photochemical quenching coefficient (qP), and non-photochemical quenching coefficient (NPQ) to varying degrees when compared to the control group (Figures 5 and 6). Although the changes in the T1 group were similar to those of the control, the T2 group exhibited a significant difference in the magnitude of decline after 24 d when compared to both the T1 group and the control.

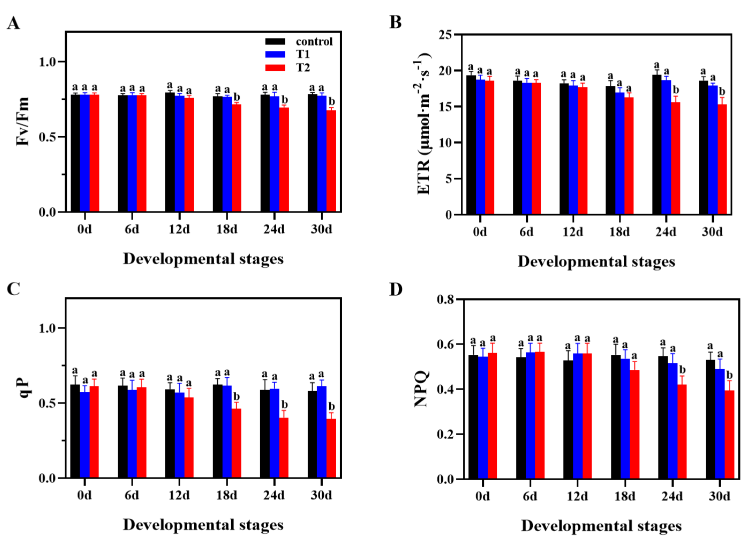


**Figure 5.** Effect of shading on chlorophyll fluorescence parameters of wax apple leaves. **(A)** Changes in Fv/Fm. **(B)** Changes in ETR. **(C)** Changes in qP. **(D)** Changes in NPQ. T1 group is 40% shading and T2 group is 90% shading. Bars with the same letters (a – b) were not significantly ($p < 0.05$) different after ANOVA test and Tukey's test

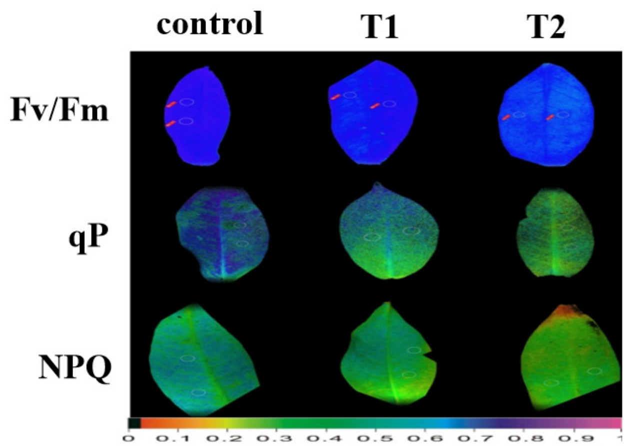


**Figure 6.** Changes in chlorophyll fluorescence parameters of wax apple leaves after 30 d of shading

## Discussion

*The impact of shading on the growth of wax apple*

Shading is a widely used method to regulate the production period of wax apple due to its simplicity and environmental friendliness. However, the effect of shading on plant growth is complex and varies among different plants. For instance, shading negatively affects the growth of *Toona sinensis*, *Cinnamomum camphora* (Liang *et al*., 2019), and *Magnolia denudata* (Wei *et al*., 2017), inhibiting their seedling height and ground diameter. On the other hand, shading promotes the growth of transplanted *super rice* (Yang *et al*., 2013) and *Solanum nigrum* L. (Du *et al*., 2012), increasing their plant height and nutritional growth.

To investigate the effect of shading on the 'Yinduhong' wax apple, we conducted an experiment using films with different light transmittance. During the experiment, it was observed that the branch growth rate increased with the days of treatment. However, the number of new shoots reached its peak at 15 d and then declined. Probably this phenomenon is due to the accumulation of nutrients in the wax apple tree. Approximately 30 new buds can emerge around 15 d, but an excessive number of buds can lead to the shedding of older ones, particularly in conditions of insufficient light. Our findings revealed that moderate shading (40% shading) did not significantly inhibit the nutritional growth of wax apple. There was no difference in the number of new shoots and branch growth compared to the control. However, heavy shading (90% shading) significantly inhibited the nutritional growth of wax apple. This indicates that heavy shading can effectively inhibit the emergence of new shoots in wax apple, reduce branch growth, and the longer the shading time, the greater the degree of inhibition on new shoot emergence.

*The impact of shading on the photosynthetic parameters of wax apple*

Shading has a direct effect on the photosynthetic rate of plants, which can be influenced by genetic traits and environmental factors such as temperature and humidity (Kawaguchi *et al*., 2021). The results showed that the Pn value of wax apple decreased as the shading intensity increased, with significantly higher Pn values observed in the control compared to the T1 and T2 groups. This suggests that normal light is essential for wax apple's energy absorption and organic matter accumulation, while shading reduces its energy absorption, resulting in weak light stress that inhibits its nutritional growth.

The decrease in plant photosynthetic rate is divided into stomatal limiting factors and non-stomatal limiting factors (Schützendübel *et al*., 2001). If the intercellular $CO_2$ concentration also decreases, it can be considered mainly due to stomatal limitation, while if the $CO_2$ concentration increases, it can be considered mainly due to non-stomatal limitation. In this experiment, the Pn, Ci, Tr, and Gs values of wax apple decreased with increasing shading intensity, indicating that stomatal limiting factors are one of the factors affecting the photosynthetic rate of wax apple leaves.

However, the photosynthetic parameters of wax apple leaves can be restored to the control level in only about 7 d after removing the shading. This suggests that the photosynthetic rate after shading can be restored through self-regulation and the subsequent production will not be affected.

*The impact of shading on the chlorophyll content of wax apple*

Chlorophyll is a crucial component in plants as it aids in the absorption, transmission, and conversion of light energy for photosynthesis (Geng *et al*., 2021). When plants are subjected to shading, they tend to increase the pigment density per unit area to absorb more light energy (Wang *et al*., 2017). In this study, the total chlorophyll content, chlorophyll a, and chlorophyll b of wax apple significantly increased compared to the control. This finding is consistent with previous research on *Robinia pseudoacacia* (Xu *et al*., 2010) and *Panax notoginseng* (Liang *et al*., 2015). Although the chlorophyll a/b ratio was generally lower in the shading group than in the control within 30 d, the difference was not significant. Some experts suggest that the increase in chlorophyll content may be linked to the reduction of photooxidative damage under weak light conditions, the increase in grana stacking degree, and grana size in chloroplasts (Atanasova *et al*., 2003).

In conclusion, the increase in chlorophyll content enhances the absorption and utilization of light energy by shaded leaves, thereby improving their adaptability to weak light environments.

*The impact of shading on the chlorophyll fluorescence parameters of wax apple*

Chlorophyll fluorescence parameters are crucial indicators for determining the stress or damage level of a plant's photosynthetic apparatus. The maximum photochemical quantum yield of PSII (Fv/Fm) is a commonly used parameter to assess plant stress, and it decreases significantly under stress conditions (Rascher *et al*., 2000). During the shading experiment, the Fv/Fm values of the 40% shading group and the control showed no significant difference within 30 d. However, the Fv/Fm value of the 90% shading group was significantly lower than that of the 40% shading group and the control, and the difference was significant after 18 d. This indicates that strong shading (90%) directly affects the photosynthetic apparatus within the leaves of wax apple, thereby achieving the regulation of its fruiting period.

The coefficient of photochemical quenching (qP) is a measure of the electron transfer activity of PSII, with a higher value indicating greater activity (Mao *et al*., 2007). On the other hand, the non-photochemical quenching coefficient (NPQ) reflects a plant's ability to dissipate excess light energy as heat (Moon *et al*., 2013). In this study, the qP and NPQ values of the control group were significantly higher than those of the 90% shading group, with a significant difference observed after 18 d. This suggests that the electron transfer activity of PSII was strongest in the control group under the experimental conditions. Shading reduces the proportion of light energy absorbed by plants for thermal dissipation, thereby decreasing the damage of light to PSII.

The efficiency of photosynthetic electron transfer (ETR) is an indicator of normal photosynthetic electron transfer (Mwelase *et al*., 2022). In this experiment, the ETR value of the shading group was initially higher than that of the control, but became lower than that of the control after 18 d. The ETR value of the 90% shading group was significantly lower than that of the control. Taken together with the results of Fv/Fm, qP, and NPQ values, it can be concluded that 90% shading has damaged the photosynthetic apparatus of plants and affected photosynthetic efficiency.

Based on the above findings, the decrease in net photosynthetic rate in the 40% shading group is mainly due to stomatal closure, while in the 90% shading group, it is due to both stomatal and non-stomatal factors.

## Conclusions

The aim of this research was to examine how shading affects the photosynthetic characteristics of wax apple leaves. The results of our study demonstrate that shading has a significant impact on the photosynthesis of wax apple leaves. When exposed to shading, wax apple leaves increase their chlorophyll content to improve their ability to absorb light. Our findings reveal that in the 40% shading treatment, the primary factor contributing to the reduction in net photosynthetic rate was stomatal limitation. However, in the 90% shading treatment, both stomatal and non-stomatal limitations were responsible for the decrease in net photosynthetic rate.

## Authors' Contributions

Conceptualization: D.S., C.H. and B.Z.; Funding acquisition: B.Z.; Investigation: D.S. and X.C.; Methodology: D.S. and B.S.; Analysed the data: X.C.; Resources: B.Z. and G.W.; Software; Supervision: Y.Y., Y.D. and S.Z.; Writing - original draft: D.S., X.C. and A.A.; Writing - review and editing: D.S., C.H., and A.A.; All authors read and approved the final manuscript.

## Ethical approval (for researches involving animals or humans)

Not applicable.

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## Conflict of Interests

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

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