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EFFECTS OF SHADING ON PHOTOSYNTHESIS CHARACTERISTICS AND SEED YIELDS OF TREEPEONY 'FENGDAN' (*PAEONIA OSTII* 'FENGDAN')

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Abstract

This study investigated the effects of shading on photosynthesis, other physiological and biochemical characteristics and seed yield of tree peony (*Paeonia ostii* FengDan).The results showed that under the natural full sunshine, the net photosynthesis (Pn) of tree peony had a mid-day depression from 12: 00pm to 4:00pm, also the total chlorophyll and the relative content of chlorophyll a and b as well as seed yield were lower compared to 25% shading condition. There was almost no mid-day depression under the condition of 25% and 50% shading, also the total chlorophyll content and the relative content of chlorophyll a and b were both higher in comparison with the full sunshine condition. Pn and seed yeild maintained a higher level all day under 25% shading. Whereas Pn had the lowest level all day under 75% shading and the yield was also declined. It is concluded that tree peony (FengDan) would grow the best and have a high yeild under the condition of 25% shading.

Key words: Tree peony 'FengDan', Shading treatment, Photosynthesis characteristics, Seed yeild.

Introduction

The oily peony (Paeoniaceae, Paeonia) has a high prolificacy, which can be used for manufacturing edible peony seed oil. It is one of the perennial deciduous shrubs with strong resistance, wide adaptation and good oil quality (Chen *et al*., 2015; Zhang, 2016), indigenous to China (Li *et al*., 2011; Zhou & Wang, 2014). At present, the oil peonies with excellent oily performance are mainly *Paeonia ostii* 'FengDan' and *Paeonia rockii* living in the region of Yangtze River and in the dry Northern area respectively (Zhou & Wang, 2014). In 2011, peony seed oil was approved as a new resource of food by the Ministry of Health of China (Ministry of health 2011).

In recent years, the peony industry started to have more important impact on local economy with a dramatic increase of cultivation area; therefore, Investigating in the steady and high yield as well as the high quality of peony oil becomes quite significant to ensure the safety of grain and oil, improve the ecological environment, increase farmers' income to help farmers to get out of poverty (Li, 2014).

Current oil peony research is mainly focused on breeding, reproduction, cultivation, resistance research, oil content analysis and comprehensive usage. In breeding, *Paeonia ostii* 'Feng Dan' and *Paeonia rockii* were chosen because of their high seed yield and oil-extracting rate; in cultivation, location selection, cultivation time, cultivation density and management were summarized based on medicinal peony production technology (Li, 2015). In reproduction, sowing is the one of the effective methods for oil peony to reproduce fast, though most of studies are still at research and development stage. Peony oil has rich fatty acids that are beneficial to human health, such as linolenic acid, linoleic acid, and oleic acid (Zhou *et al*., 2009; Wang *et al*., 2009; Deng *et al*., 2010; You *et al*., 2011; Kang *et al*., 2013; Li *et al*., 2013).

Though light is the primary energy source in plant photosynthesis(Kalariya *et al*., 2013), but excess light can also restrain photosynthesis (Wang *et al*., 2002; Bertaminia *et al*., 2006; Cavagnaro & Trione, 2007; Dai *et al*., 2009; Zhou *et al*., 2010; Cai, 2011). Ornamental peony research has shown that appropriate shading can promote growth and flowering (Zhu *et al*., 2012; Zhao & Hao., 2012). The optimum conditions for oily peony are forests and forest gap environment with appropriate shading (Zhang *et al*., 2014). However, the impact of the different light intensity on oily peony growth and seed yield had rarely been studied.

This study adopted the artificial lighting method to explore the impacts of different light intensities on peony growth, photosynthetic characteristics, physiological and biochemical characteristics and the seed oil production. In this study we have also found the optimum light intensity for peony oil cultivation, providing the foundation for the high peony seed oil yield and interplanting tree peony in forests.

Materials and Methods

The experimented tree peonies (*Paeonia ostii* FengDan) are normally grown for four years in a tree peony field, located in the northwest agriculture and forestry science experiment fields in Yang Ling, Shaanxi Province, China (north latitude 34°16'56", 4, east longitude +108°4'27"). The area belongs to the warm temperate semi-humid continental monsoon climate, with a 12.9°C annual average temperature, a 211 days frost-free period, and a 635.1 mm annual average rainfall. Four treatment groups were set up for testing, each group included 30 plants: In group 0, plants were exposed to the sun directly (with 0% shading), it was set as a control group. In group 1, plants were covered with one layer of two stitches shade net (with 25% shading). In group 2, plants were covered with one layer of three stitches shade net (with 50% shading). In group 3 plants were covered with two layer of shade three stitches net (with 75% shading). The experiment was repeated thrice and the peony plants were properly managed and maintained during the experiments.

Growth index determination: The vegetative growth indexes of peony plants were observed and measured, including plant height, crown breadth, the number of sections, the increment of sections and number of flower buds. 10 plants were selected for each process, where plant height, crown breadth and flower numbers were measured during flowering period and pod size, pod number and seed number in each pod were measured during ripening period.

Photosynthetic parameters measurement: The photosynthetic parameters of peony in the flowering period and early stage of seed maturing period were determined by Li - 6400 photosynthetic apparatus on healthy leaves with red/blue lighting. The light response curves measurements were performed between 10:00 am-12:30 pm on a sunny day, using red and blue lighting, with the active photosynthetic radiation set to 2000, 1600, 1200, 100, 800, 650, 500, 350, 200, 150, 100, 25, 50, and 0μmol/(m2·s). The leaves chosen were on the top center of the plant to ensure sufficient sunlight and to eliminate diurnal variation. Other indexes such as net photosynthetic rate (Pn), gas conductance (Gs), intercellular CO_2 concentration (Ci) and light intensity (Pi) were measured once every two hours from 8:00am-6:00pm on the same day. By using a standard leave room, the plant photosynthetic diurnal curve was obtained. For each treatment, 3 plants with two leaves on each were selected. By repeating the process twice on each leaf, the averages were calculated.

Chlorophyll content measurement: Top leaves from two plants were collected during fruit ripping period. After cleaning and drying the fresh leaves, 0.05g leaves with midrib removed were measured with the electronic analytical balance. The leaves were then cut into filaments of 1mm and put into a 10ml test tube. After adding 10ml mix of Acetone and Ethanol (90%) with a 1:1 ratio, the test tube was sealed immediately and stored in dark for 24 hours at room temperature. After mesophyll tissue becoming transparent completely, the wavelengths of 645 nm and 663 nm were measured with ultraviolet spectrophotometer (UV-2450). Finally, the chlorophyll a, b and total chlorophyll contents were calculated with the formula: Eq. (1) C_a=0.0127A_{663}-0.00269A_{645} Eq. (2) C_b=0.0229A_{645}-0.00468A_{663}.

Seed production measurement: After most of the fruits matured, some fruits were cut off and their total weight, the number of pod and the number of seed per pod were measured. After separating the seeds and pods, the weight of thousands of seeds and the output per area were determined. Each group was separated when performing statistical measurements, and the single factor analysis of variance was carried out.

Statistical analysis: Treatments were arranged in a randomized complete block design with three replications. SPSS19.0 was used to analyze the single factor variance and correlation analysis.

Results

The influence of light intensity on peony growing: From table 1, six terms including the number of sections, the crown breadth, the largest stem growth, the number of flower buds, the number of pod and the pod length were all reduced by shading. Plant height was reduced by medium shading but increased by weak lighting. With 0-100% shading, the maximum difference between plant heights, crown breadth, number of new branches and the maximum stem growth were 1.2cm, 5.2 cm, 0.2 and 3.5 cm respectively. The maximum difference between the flower bud number per plant was 1.3, between the pod number per plant was 1.8 and between the pod length was 1.1 cm. For oil peonies, Shading had a significant effect on the maximum stem growth, but not on the growth form (Table 1).

The effects of light intensity on the photosynthetic characteristics of peonies: By growing under different lightings for a long time, the characters of leaves changed in response to light. According to Fig. 1, during June and July, the natural light intensity (PAR) was up to 1713.771μmolm^{-2} s^{-1} at noon in Yangling region. Under 25%, 50% and 75% shading conditions, the maximum light intensity during the day was 1309.742, 680.809 and 306.480μmol m^{-2} s^{-1} respectively (Fig. 1).

Table 1. Growth indexes under different illumination conditions of tree peony ‘Feng–Dan’.

Light condition	Plant height (cm)	Crown breadth (cm)	Number of sections (per plant)	The sections increment (cm)
0% Shading	67.8 ± 13.3a	44.2 ± 9.6a	1.4 ± 0.8a	22.4 ± 4.1a
25% Shading	67.3 ± 14.5a	39.1 ± 12.8a	1.5 ± 0.6a	18.9 ± 5.7b
50% Shading	67.2 ± 15.8a	41.4 ± 10.4a	1.3 ± 0.7a	21.4 ± 5.2ab
75% Shading	68.4 ± 11.2a	42.2 ± 11.4a	1.3 ± 0.5a	20.6 ± 5.8ab

75 % equal 75 % shading or % 75 light this is not understood

Light condition	Number of flower buds (per plant)	Number of pod (per plant)	Length of pod (cm)
0% Shading	1.5 ± 0.8a	4.8 ± 3.4a	3.1 ± 1.8a
25% Shading	1.6 ± 0.8a	4.0 ± 3.3a	2.4 ± 1.8a
50% Shading	1.4 ± 0.8a	4.0 ± 3.9a	2.5 ± 2.0a
75% Shading	1.3 ± 0.8a	3.0 ± 3.2a	2.0 ± 2.1a

According to Fig. 2, peony's light response curve trend was consistent with different light conditions indicating that the peony leaf had light saturation when light intensity was enough (about 1500μmolm^{-2} s^{-1}). The maximum net photosynthetic rate of peonies was 11.15, 10.36, 8.99 and 5.82 for 25%, 50%, 75% and 0% shading conditions respectively (Fig. 2).

With the full light condition, the diurnal variation of the peony's leaf showed an obvious "noon break" phenomenon (Fig. 3). From 8:00 to 9:00, the Pn was the highest (7.42 μmol CO_2·m^{-2}s^{-1}), then it began to decline. After reaching the minimum (2166μmol CO_2·m^{-2}s^{-1}) at around 14:00, it started to rise again. A small peak appeared at about 16:00 and then it decreased again. With 25% and 50% shading conditions, Pn declined around 14:00 slightly. However, Pn had no evidence of dropping at noon for the rest lighting treatments. Compared to the full light processing, the minimum Pn concentration were 162%, 146% and 84% of 25%, 50% and 75% shading conditions respectively. Pn had maintained a relatively high level during the day with 25% shading, whereas the Pn concentration was lower with 50% shading and was the lowest with 75% shading (Fig. 3).

The Ci concentration of peony leaves decreased from 8:00 to 10:00 and; then it began to increase under the full light processing condition (Fig. 4). After reaching the maximum value around 12:00, it started to drop again. The minimum value appeared around 16:00 and then it rose again. With 25% and 50% shading conditions, the Ci concentration change appeared to have a similar trend, except for with 25% shading, the second rise of Ci occurred slightly earlier compare to natural light and 50% shading conditions. Ci declined from 8:00 to 13:00 with 25%shading condition, and rose slowly from 13:00 to 18:00. The Ci concentration of peony leaves with 75% shading was significantly lower than that of 25% and 50% shading (Fig. 4).

Under 25%, 50% and 0%shading conditions, the stomatal conductance (Gs) of peony leaves rose rapidly from 8:00 to 10:00, then dropped rapidly to a lower level from 10:00 to 12:00; A small peak appeared around 14:00 after rising slowly-and then it decreased again (Fig. 5). Whereas with75% shading, Gs declined slowly from 8:00am to 18:00, and-the Gs of peony leave with 75% shading was significantly lower than that with the other processing conditions. The Gs of peony leaves during the day had a decreasing trend with 0%, 50% and 25% shading conditions (Fig. 5).

The transpiration rate (Tr) of peony leaf rose slowly from 8:00 to10:00 under the full light and the 25% shading conditions (Fig. 6), then it started to decline. After, the minimum value occurred at 12:00, it started to increase, and after reaching a small peak at 14:00 it dropped again. Whereas, under 50% and 75% shading conditions, Tr rose from 8:00 to 14:00 and then dropped continuously (Fig. 6).

The influence of light intensity on the peony leaf chlorophyll: Chlorophyll is the main source for plant photosynthesis to take place. The content and the composition of Chlorophyll in leaves are directly related to the photosynthetic rate. The content of chlorophyll a, b and the total were significantly different under the different illumination conditions, where the segmental light conditions all gave higher values than the natural lighting condition (Table 3). Also, with the declining of the light conditions, the relative content of chlorophyll a, b and the total was increased. Compared with the 0% shading, the total chlorophyll content was increased 16.64%, 24.56% and 55.44% with 25%, 50%, 75% shading respectively. The relative content of chlorophyll a was increased by 15.90%, 21.79% and 51.73% respectively and the relative content of chlorophyll b was increased by 18.68%, 32.19% and 65.66% respectively. In conclusion, shading increased the content of chlorophyll a, chlorophyll b and the total chlorophyll of *Paeonia ostii* 'FengDan' (Table 2).

The light intensity on the behavior of the peony fruit and seed production: Table 3 showed that with different shading conditions, the number of carpel and grain per carpel did not show significant difference. The mean for the number of carpel and grain per carpel were 4.72 and 3.84 respectively. However, the total fruits, seeds, seed weight and fruit total weight were significantly different under different lighting conditions. The highest value at 25% shading was nearly the double of the lowest value, which occurred at 75% shading. The highest values for the seed yield were 5457.34 g//hm^2 for 25% shading and 5441.42 g//hm^2 for 0% shading. They were almost twice of the lowest ones, which was 2815.37 g/hm^2 at 75% shading. The variance of seed yield with 75% shading was smaller than other lighting conditions, which was easier to achieve a stable and high yield (Table 3).

Discussion

The results showed that, both full lighting (0% shading) and excessive shading (75% shading) could prevent tree peonies from growing and developing well, whereas moderate shading was advantageous. Compared to 50% shading, the growth and development of the tree peony under 25% shading treatment was better. The results were consistent with the research conducted by Zhang *et al*., (2014), as shown in the following respects.

Plant height, crown breadth and the flower numbers could directly indicate the growth situation of the peony plant (Zhao & Hao, 2012). The experiment results showed, shading reduced the number of sections, crown breadth, stem growth, flower buds, pod and pod length. In average, medium shading (50%) reduced the plant height, but excessive shading (75%) increased plant height. The effect of shading, however, on the growth situation of the peony plant between different groups was not significant (p<0.05) considering that illumination intensity was not the essential factor in peony's vegetative period, which was similar with the result researched by Zuo (2009).

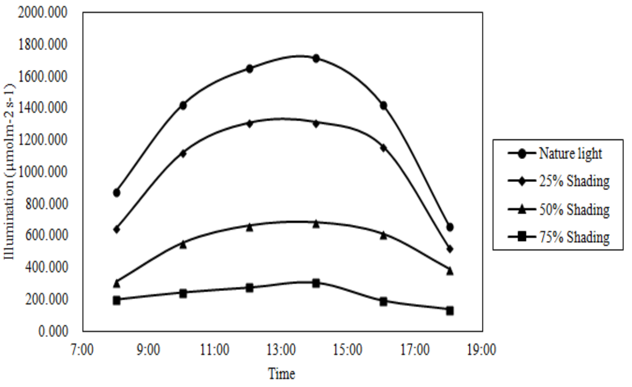

Fig. 1. Illumination variation under different processing conditions.

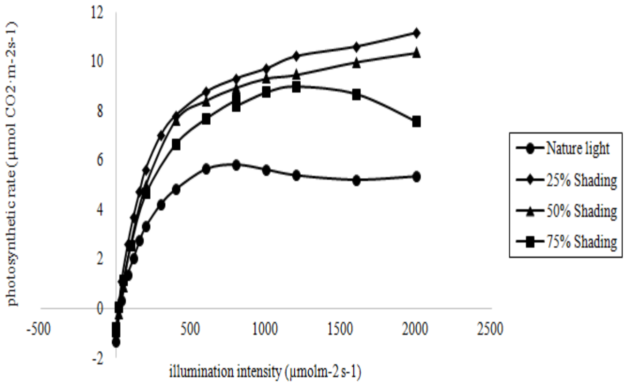

Fig. 2. The light response curves of tree peony 'FengDan' under different Illuminations.

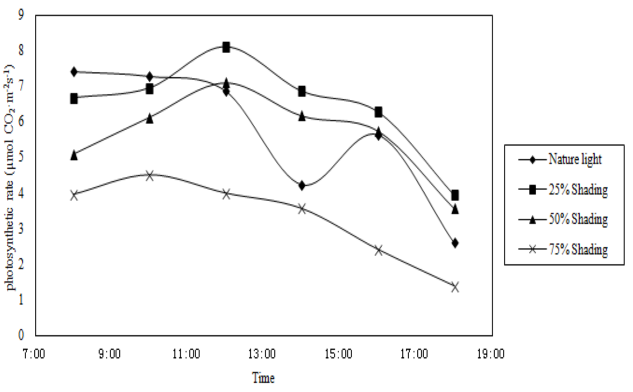

Fig. 3. The diurnal change of photosynthetic rate under of tree peony 'FengDan' under different Illuminations.

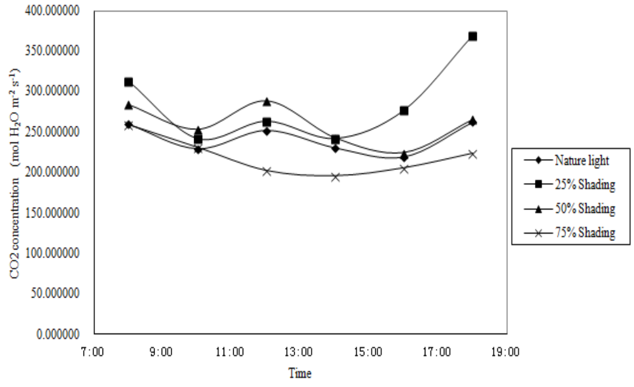

Fig. 4. The intercellular CO_2 concentration of tree peony 'FengDan.

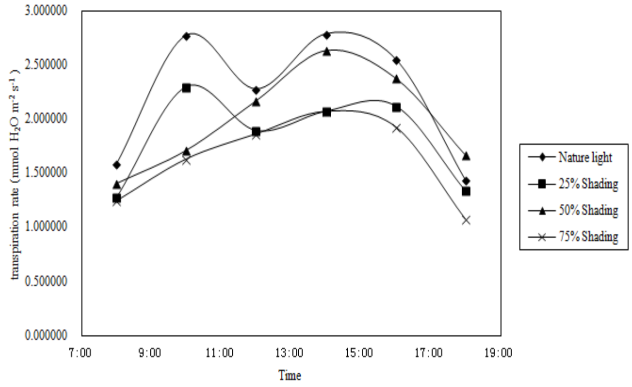

Fig. 5. The daily variation of transpiration rate of tree peony 'FengDan'.

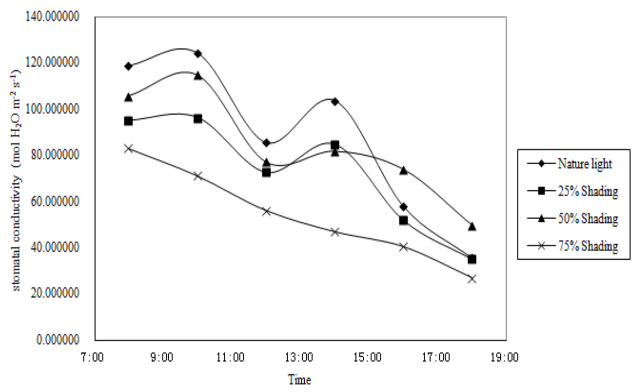

Fig. 6. The stomatal conductivity of tree peony 'FengDan'.

Photosynthesis is one of the basic activities of plants, which produces essential substances to maintain their life activities (Ouzounidou *et al.*, 2012). Pn is directly related to the accumulation speed of plant photosynthetic products (Bertaminia *et al.*, 2006; Cavagnaro & Trione, 2007; Wang *et al*., 2011). In the experiment, with natural light processing, the minimum Pn value occurred when the light intensity was the highest, then the Pn content started to increase and had a small peak in afternoon, which was also reported in the previous research in other kinds of peony (Li *et al*., 2016).Such phenomenon is called "photosynthetic noon break", which is common for many plants, such as *Ligustrum vicaryi*, *Ligustrum quihoui* and various landscape tree species. (Zhuang *et al*., 2007; Zhang *et al*., 2009; Gao, 2016). In shading processes, Pn hardly had above characteristics instead, the Pn daily curve had only one peak, with increasing from 8:00am to 12:00pm and declining from 12:00pm to 4:00pm. The similar phenomenon was reported by Cai *et al*., (2016) in previous study. When photosynthetic rate decreases with the intercellular CO_2 concentration (Ci) rises, the main reason is non-stomatal, but the decline of mesophyll cells photosynthetic activity (Caemmerer & Farquhar 1981; Farquhar & Sharkey, 1982). Fig. 3 also shows that the change of Pn and Ci are not synchronized. Therefore, we can infer that with natural lighting, the decline of Pn is caused by the intense light at noon but not the decrease of Ci. With 25% shading, the Pn content maintained at a relative high level even though Ci decreased at noon, which is beneficial for peony to accumulate more photosynthetic product. In comparison, with 75% shading, Pn content was very low and with 50% shading Pn content stayed in between that of 75% and 25% shading. In natural lighting, although Pn was higher at the beginning, it reduced significantly at noon because of the lunch break phenomenon which lead to a significant decrease for the peony photosynthetic product. Besides, the stomata play critical roles in photosynthesis and leaf gas exchange, which are very important for the effectiveness of crop plants. There are many factors impacting the stomata movements, such as light intensity and quality, temperature, relative humidity, CO_2 concentration, etc. (Taiz & Zeiger, 2008; Casson & Gray, 2008). Similarly in present study, growing stomal conductance was observed with increasing light intensity (Özer, 2017).

Plant light saturation point (LSP) and the size of the compensation point (LCP) could reflect weak light utilization ability of the plants, where lower light compensation point leads to a higher photosynthesis accumulation under the weak light (Liu *et al.*, 2007; Ye & Zhao 2010). The light response curve showed an obvious difference between light saturation point and the light compensation point. The highest light saturation point occurred when the plant was 25% shaded where the light was most efficiently utilized. The lowest light saturation point occurred with the natural light condition where the light utilization efficiency was the lowest. The possible reason was that the measurements were taken during the "noon break". In conclusion, the tree peony *Paeonia ostii* 'FengDan' had a high adaptability to weak light because the light compensation point was relatively low under all lighting conditions. Similar findings were also reported for different plant species, particularly in the shade-tolerance plants (Zhu *et al*., 2013; Huang *et al*., 2014; Li *et al*., 2016).

Chlorophyll is essential for photosynthesis. Chlorophyll includes both chlorophyll a and chlorophyll b, where the former mainly uses short waves and the latter mainly absorbs long waves for photosynthesis (Atanasova *et al.*, 2003; Lichtenthaler *et al.*, 2007; Wang *et al*., 2011). The experiment results showed that the reduced light increased total chlorophyll content and the relative content of chlorophyll a and b, which lead to a conclusion that shade could motivate the peony to utilize more energy as well as increase its ability to use short waves. In previous studies, the trend of total chlorophyll change with the shading growing was basically consistent (Zhou *et al*., 2010). Similar results were also reported for different plant species (Wang *et al*., 2011; Özer, 2017).

The peony seed yield in this experiment was relatively low (2815.37 ~ 5457.34 g/hm^2) compared with the similar studies by Li (2014) and Song *et al*., (2018). There were three possible reasons: firstly, the peonies were in the recovery period, which were transplanted for two years, and the beginning period of fruits (Li, 2014; Yin *et al*., 2018); secondly, the growth of peony was affected since the intercrop occurred during the peony seed maturation period. Finally, the planting density in this experiment is lower than that in other similar ones. Lighting condition had little impact on fruits and seeds number per carpel, but it had significant impact on the seed yield, similar results were obtained compared to other impact factors in peony growth, such as planting density (Wang *et al*., 2018) and Canopy density in forest (Song *et al*., 2018). It showed that the natural lighting condition and weak lighting condition could always reduce the peony seed yield, whereas the peony seed yield would be the highest with 25% shading condition.

In conclusion, applying 25% shading to peonies is not only beneficial to their growth, but also could increase their seed yield, thus could greatly improve the economic value of tree peony 'FengDan'. Therefore, it is suggested they may be interplanted with other economic crops, such as *Xanthoceras sorbifolia* (Sun *et al*., 2016), *Acer truncatum* (Wei *et al*., 2018) to improve the land utilization rate and the overall economic value.

Table 2. The influence of the content of chlorophyll a, b and total in different illumination of tree peony 'Feng–Dan'.

Light condition	Ca (mg/L)	Cb (mg/L)	CT (mg/L)
0% shading	5.578 ± 1.747b	2.023 ± 0.618b	7.601 ± 2.353b
25% shading	6.465 ± 1.403ab	2.401 ± 0.403b	8.866 ± 1.774b
50% shading	6.794 ± 1.954ab	2.674 ± 0.697ab	9.468 ± 2.625ab
75% shading	8.464 ± 1.686a	3.351 ± 0.674a	11.815 ± 2.354a

Table 3. The influence of seed yield and so on in different illumination of tree peony 'Feng– Dan'.

Light condition	Carpel number	grain number per carpel (grain)	Total fruits	Total seeds (grain)
0% Shading	4.95 ± 0.04a	3.99 ± 0.83a	14.00 ± 3.61a	243.33 ± 45.63a
25% Shading	4.68 ± 0.27a	4.04 ± 0.76a	13.33 ± 3.06a	247.67 ± 47.37a
50% Shading	4.72 ± 0.13a	4.01 ± 0.87a	10.6 ± 3.06ab	171.6 ± 56.50ab
75% Shading	4.50 ± 0.46a	3.30 ± 0.75a	7.33 ± 1.53b	119.33 ± 14.01b

Light condition	Seed weight (g)	Fruit total weight (g)	Seed yield (g/hm^2)
0% shading	72.97 ± 9.61a	112.97 ± 26.64a	5441.42 ± 942.82a
25% shading	83.3 ± 15.58a	123.33 ± 29.11a	5457.34 ± 537.73a
50% shading	64.5 ± 15.63a	101.2 ± 26.29ab	3794.78 ± 904.13b
75% shading	39.66 ± 7.39b	63.00 ± 13.16b	2815.37 ± 347.24b

Note: Using Duncan's multiple range test and the test analysis of variance, different letters indicate significant differences in the same column (p<0.05). The values represent the means ± SD

Abbreviations: Pn-net photosynthetic rate; Gs-gas conductance; Ci-intercellular CO_2 concentration; Pi-light intensity; Tr-transpiration rate.

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