RESEARCH ARTICLE

Effects of different canopy layers on leaf photosynthetic characteristics of apple trees

Ke Huang* and Hongbing Fu

School of Biological and Environmental Engineering, Xi'an University, Xi'an 710065, Shaanxi Province, China.

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The overall photosynthetic capacity of apple tree canopy is the main factor determining apple tree productivity. Therefore, the effects of different canopy layers on the leaf photosynthetic characteristics of apple trees were studied. From 10-year-old Red Fuji apple orchard, three forms of apple trees named as evacuation stratification apple tree, natural round head apple tree and improved spindle apple tree were selected as the study objectives to measure and compare the seasonal variation of canopy characteristics and leaf quality. The effects of spraying uniconazole (S_{3307}) and Diethylaminoethylhexanoate (DTA-6) on leaf chlorophyll content and net photosynthetic rate of apple trees in different canopy layers were investigated. The difference of light response curves, photosynthetic characteristics, and their correlation to leaves from the surface, middle, and lower canopy layers were studied. The results showed that evacuation stratification form had the best canopy ventilation effect and leaf quality, followed by the improved spindle form. Comparing to the leaf surface sprayed with water (control group), S_{3307}, and DTA-6 treatments increased leaf chlorophyll content and net photosynthetic rate in different canopy layers of apple trees. The net photosynthetic rates of the upper and middle layer leaves treated with plant growth regulator were greatly improved with DTA-6 treatment group higher than that in S_{3307} treatment group. The leaf photosynthetic rate was much higher in the crown layer than that in the middle and lower layers, which changed obviously with the change of light intensity. The photosynthetic indexes of apple canopy leaves including light saturation rate, stomatal conductance (Gs), intercellular CO_2 concentration, transpiration rate (Tr), and photosynthetic nitrogen efficiency were the highest in the surface leaf followed by the middle layer, and then, the lower layer. However, an extremely significant positive correlation was observed between Tr and Gs. Therefore, apple tree productivity can be improved by adjusting the apple tree canopy to increase the leaf photosynthetic capacity.

Keywords: apple tree; canopy layers; leaves; photosynthetic characteristics; chlorophyll.

***Corresponding author:** Ke Huang, School of Biological and Environmental Engineering, Xi'an University, Xi'an 710065, Shaanxi Province, China. Email: hk02102022@126.com.

Introduction

Apple, as one of the four major fruits in the world, ranks the first in terms of cultivation area and total output in China. In recent years, for high-yield apple cultivation, close-planting mode is often adopted. Due to large branch volume, closed canopy, and insufficient light and ventilation in the inner chamber, fruit yield and quality are low [1-2]. Therefore, improvement of light and ventilation in the canopy, selection of reasonable canopy structure, and screening plant growth regulators are important and urgent issues in apple production and cultivation [3].

Photosynthesis is the process by which green plants use light energy through chloroplasts to convert carbon dioxide and water into energy-storing organic matter and release oxygen [4]. Photosynthesis is the main channel for plants to access nutrients. Leaves produce organic substances through photosynthesis to meet the needs of plant growth and development, and finally plant yield [5, 6]. Believed that the yield potential was also limited by the material production capacity of the population, and building a good population canopy structure could improve the photosynthetic efficiency of plants and lay a foundation for the high yield of plants. It has been known that chemical control technology has been constantly applied to plant production practice, which can increase plant yield by improving photosynthetic efficiency and regulating physiological metabolism of plants. Therefore, it is necessary to improve the photosynthetic efficiency of leaves in different apple tree canopy layers and to enhance the material production capacity of leaves using exogenous substances, which is of great significance for increasing apple production.

It has been reported that uniconazole (S_{3307}) treatment significantly increased the photosynthetic area of the upper, middle, and lower layer leaves of 'Yayu No. 2' maize, and the upper layer leaves had the largest increase. Meanwhile, S_{3307} treatment also significantly improved the net photosynthetic rate, transpiration rate, and stomatal conductance of ear leaves during the days to silking [7]. The yield and quality of fruit trees are not only related to the photosynthetic characteristics of individual leaves, but also may be closely related to the photosynthetic characteristics of the entire canopy leaf population. DTA-6 can effectively improve the root reduction of plants, promote the growth and regulation of plants, and indirectly affect the formation of plant photosynthesis [8, 9].

In recent years, researchers have conducted excessive studies on plant photosynthetic characteristics such as different growth stages, leaf position, variety differences, diurnal variation, annual variation characteristics, *etc*. However, most studies focused on the crown surface leaves with few studies on the middle and lower layer leaves [10]. In this study, three forms of apple trees' characteristic parameters of leaf photosynthetic capacity in different canopy layers, seasonal variation rules of canopy characteristics, leaf quality and regulation effects of plant growth regulators S_{3307} and DTA-6 on leaf photosynthetic characteristics of different canopy layers were investigated, which could strengthen the overall understanding of photosynthesis in apple trees and provided a theoretical basis for building ideal high-yield population model and canopy structure of apple trees.

Materials and methods

Apple tree leaf treatment and sample collection

Three experimental plots with a perennial supply of 474 kg/hm^2 of pure nitrogen were selected for this study. The plot area was 105 m^2 including 6 of 5 meter long rows with a plant spacing of 3.0 m, row spacing of 3.5 m, and 1 meter corridor. The apple tree variety was 10-year-old Fuji. Total 15 trees including 5 of evacuation and layering form, 5 of natural round head form, and 5 improved spindle shape form were employed. S_{3307} and DTA-6 were applied to treat experimental apple tree leaves with the optimal treatment concentrations of 50 and 60 mg/L, respectively. Water in a dosage of 225 L/hm^2 was sprayed on the leaf surface as control (CK). The treatment reagents were sprayed at about 16:00 pm on a sunny day in the early flowering period (R1) without wind. 15 apple tree branches were randomly selected from each sample plot, and the photosynthetic effective radiation (PAR) of leaves was measured from top to bottom with LI-250A optical quantum density detector (LI-COR Corporation, Lincoln, NE, USA). The light quantum density received by the first mature leaf at the top of the branch was set as 100%, and the light absorption rate of each leaf was then calculated in turn. According to the light absorption rates of 100%, 50-70%, and 0-15%, the apple tree crown was divided into three canopy layers named as the surface layer, the middle layer, and the lower layer. Healthy matured leaves with similar color, shape, and position from each canopy of apple tree were randomly collected in the middle of the new shoots from mid-late April to mid-late August.

Apple tree canopy stratification

With the trunk of the tree body as the axis, a bamboo pole was used to form a cube (3 × 3 × 3 m^3), which was additionally divided into 0.5 × 0.5 × 0.5 m^3 small cubes with 75 cm as a layer. From the first main branch of the lower part of the tree body upward, it was divided into three layers in turn.

Determination of seasonal variation rules of canopy characteristics

From the middle of April to August, the canopy structure of the tree body was determined by using LAI-2000 canopy analyzer (LI-COR, Lincoln, NE, USA). In the case of no shelter, the 90° lens cover was used to measure a value of the sky as the control value A. Then, a value was measured in each of the four directions around the tree trunk as value B. The measurement was repeated for 3 times. The measured data were analyzed by using FV-2000 software (LI-COR, Lincoln, NE, USA) to determine leaf density and sky visibility [11].

Determination of leaf quality

In mid-June, 10 complete apple tree leaves were randomly selected from the middle part of new shoots. The chlorophyll content was determined by acetone extraction method. Briefly, 0.1 g of fresh leaves was grinded with 80% acetone to make a homogenate before brought up to the total volume of 10 mL with 80% acetone. The leaf water content and specific leaf weight were determined by drying the leaves in an air circulation place or an 80℃ oven. The leaf thickness was measured by using vernier caliper.

Determination of photosynthetic indexes

In late May, the light response curve of leaves was measured by using LI-6400 portable photosynthesis measurement system (LI-COR Corporation, Lincoln, NE, USA) from 9:00 am to 11:30 am on a sunny and less cloudy day. The light intensity gradient of the surface leaf was set as 1,800, 1,400, 1,000, 800, 500, 200, 100, 50, 20, and 0 $\mu mol/m^2 \cdot s$, while the middle layer was set as 1,400, 1,000, 800, 500, 200, 100, 50, 20, and 0 $\mu mol/m^2 \cdot s$, and the lower layer was set as 1,000, 800, 500, 200, 100, 50, 20, and 0 $\mu mol/m^2 \cdot s$. The light saturation points of each canopy leaf were determined according to the measurement results and relevant indexes of leaves in each canopy layer were then determined including light saturation rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), transpiration rate (Tr), and related ecological indexes such as CO_2 concentration (CO_2R), leaf temperature (Temp), *etc*. Four matured leaves were selected from each canopy layer of apple trees in each row for measurement. After the measurement, the measured leaf was placed in liquid nitrogen and transferred to -80°C for storage [12].

At 3, 11, 21, 36, and 46 days after spraying plant growth regulator, the canopy was divided into upper, middle, and lower layers according to the number of plant nodes. Leaves with fixed nodes were selected from each layer, and 5 representative apple leaves were selected from each treatment plot. The chlorophyll content of different canopy layers was determined by SPAD-502 chlorophyll analyzer (Konica Minolta, Marunouchi, Japan).

Measurement of physiological indexes

20 healthy matured leaves with similar leaf color, shape, and position were selected from each canopy layer of apple trees in each row and were sampled with a hole punch. The samples were baked to constant weight at 70°C to calculate specific leaf weight (LMA). The dried sample was ground, and the total carbon (C_M) and nitrogen (N_M) contents per unit leaf weight (mol/kg) were measured by using vario Max cube organic elemental analyzer (Elementar, Langenselbold,

Germany), and then, were converted into the content per unit leaf area (C_A and N_A) (mol/m^2) by specific leaf weight. Photosynthetic nitrogen efficiency (PNUE) was calculated based on light saturation rate (Pn) and unit area nitrogen content (N_A) (PNUE = Pn / N_A). Fresh leaf chlorophyll (chl) was determined by mixing leaf sample with N, N- dimethylformamide (DMF) solution in a dark environment at 4°C overnight and measuring the absorbance of the solution by using ultraviolet spectrophotometry (Unic, Shanghai, China).

Statistical analysis

Excel 2010 software (Microsoft, Redmond, WA, USA) was used for statistical analysis and mapping of data. SPSS 19.0 (IBM, Armonk, NY, USA) was used for one-way analysis of variance, and Duncan's method was used for multiple comparison and significance test [13].

Results and discussion

Seasonal changes in canopy characteristics of the three apple tree forms

The growth and development of apple tree's branches and leaves are closely related to the seasonal changes in sky visibility and leaf density. Between April and July, the density of apple tree leaves gradually increase and are stabilized, while the sky visibility gradually decreases. The sky visibility of the three tree's forms reached its peak in June with the highest sky visibility of 13.3% from evacuated hierarchical tree form. During the growth period of apple tree's branches and leaves, the evacuation and layering form of the canopy has the best ventilation and light transmittance followed by the improved spindle shape, and then, the natural round head shape. Therefore, the higher the sky visibility reached, the better the photosynthetic absorption achieved, and the higher the light transmittance reached, the better the growth of apple realized.

Difference in canopy leaf quality of the three apple tree forms

The chlorophyll content in the middle and lower crown leaves of the modified spindle shape was significantly higher than that of the open laminated and natural round head shapes. The specific leaf weight of the three tree forms differed significantly in the lower canopy with the improved spindle form having the largest specific leaf weight. In terms of leaves, the water content of the natural round head form in both upper and lower canopy leaves was significantly higher than that of the evacuation and the spindle forms. In the middle canopy layer, the water content of modified spindle shaped leaves was significantly higher than that of natural round headed leaves. The chlorophyll content in the crown leaves of the three tree forms gradually decreased from the lower layer to the upper layer. In addition, the specific leaf weight of evacuation and natural round head canopy gradually increased from the lower layer to the upper layer, while that of the improved spindle form decreased in the order of upper layer > lower layer > middle layer. In summary, the quality of canopy leaves in the form of evacuation and stratification was the best followed by the improved spindle shape. The quality of canopy leaves in the form of natural round heads was the worst.

Effects of S3307 and DTA-6 on leaves in different canopy layers of apple trees

After spraying plant growth regulators, the chlorophyll content of apple tree leaves first increased, and then, decreased with the extension of processing time. In the comparison of different canopy layers, the chlorophyll content of apple tree leaves was arranged in descending order from the upper layer to the middle layer, and then, to the lower layer (Figure 1). The chlorophyll content in the upper layer of the 10-year-old red Fuji apple tree reached its maximum at the 36th day, while the chlorophyll content in the lower and middle layers reached its maximum at 20th day. Comparing to the control group, the chlorophyll content in the upper and lower layers of DTA-6 treatment trees increased, and the chlorophyll content in the middle and lower layers of S3307 treatment trees increased by 5% and 11%, respectively. After

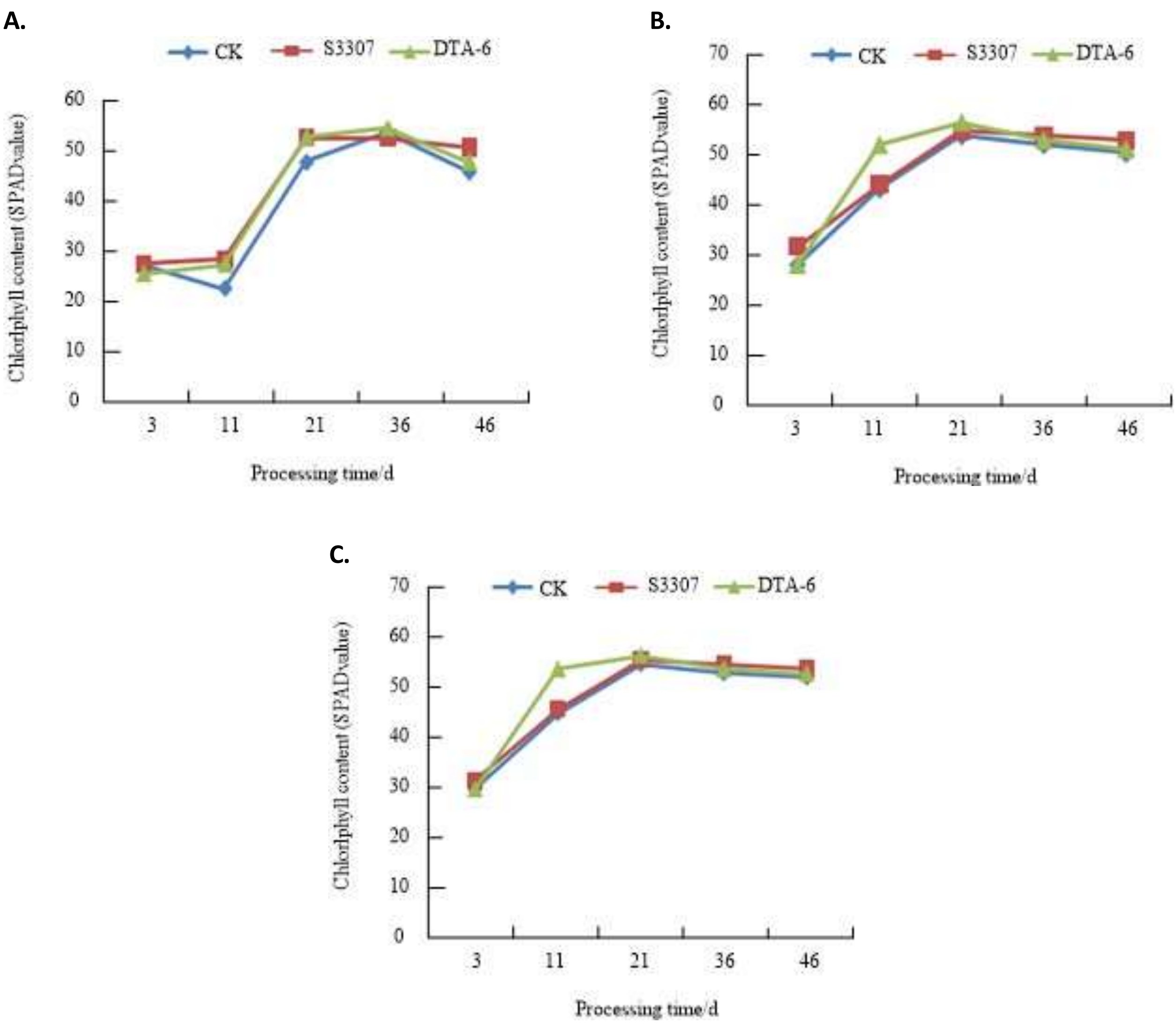

Figure 1. Effects of plant growth regulators, S_{3307} and DTA-6, on chlorophyll content of apple tree leaves in supper layer (A), middle layer (B), and lower layer (C) of canopy. (CK: control group)

applying plant growth regulators at 20, 35, and 45 days, the chlorophyll content in the lower layer of DTA-6 treatment trees increased comparing to the control group, and the chlorophyll content in the lower layer of S3307 treatment trees was generally higher than that in the control group at all stages. The chlorophyll content in the middle layer leaves of 10 year old red Fuji apple tree treated with plant growth regulators was generally higher than that in control group.

The effect of plant growth regulators on the overall net photosynthetic rate of plants was greater in the DTA-6 treatment than in the S3307 treatment. The net photosynthetic rate of the upper and middle layer leaves significantly increased after treatment (Figure 2). After 3, 11, 21, and 56 days of the treatment with both plant growth regulators, the net photosynthetic rate of the upper layer leaves increased. The net photosynthetic rate of the lower layer leaves reached the peak after 46 days of the treatment. After 3 days of the DTA-6 treatment, the net photosynthetic rates of the upper and lower layer leaves were lower than that of the control. The maximum net photosynthetic rates of the upper and middle layer leaves were 75% and 22%

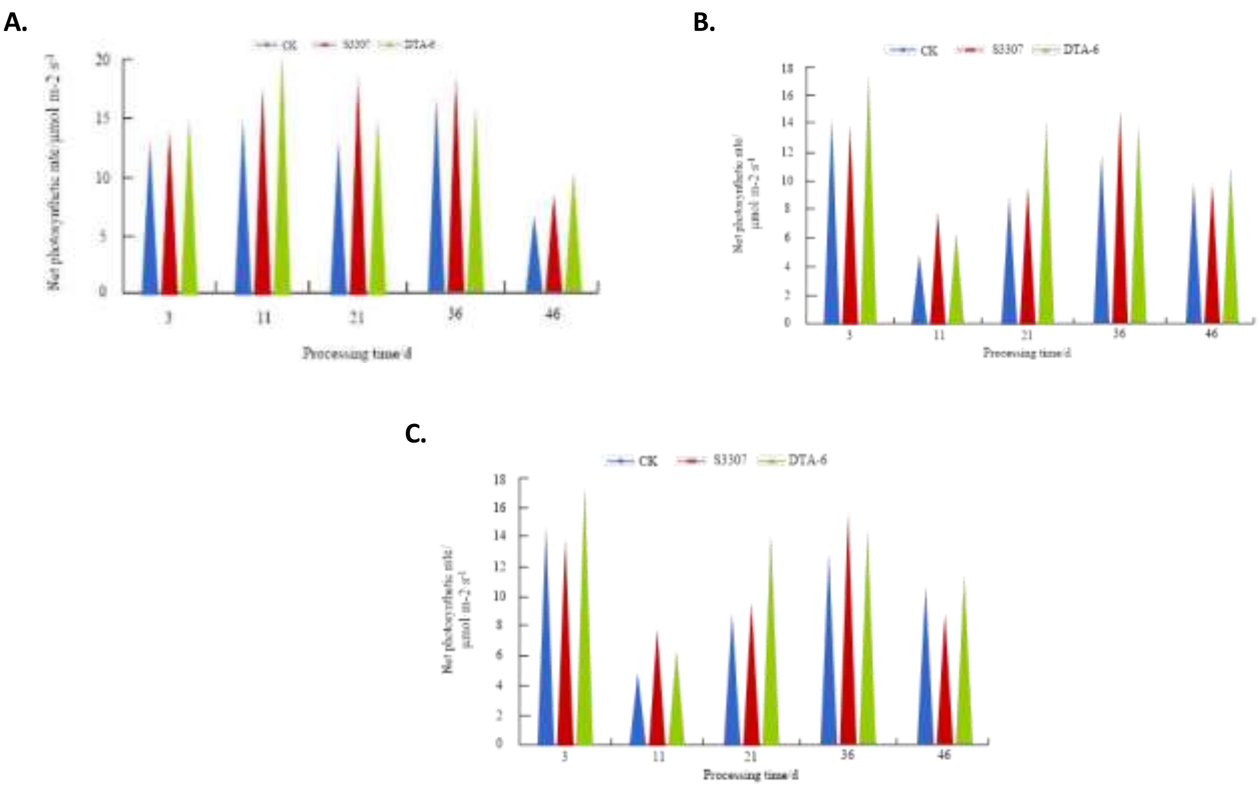

Figure 2. Effects of plant growth regulators, S3307 and DTA-6, on net photosynthetic rate of apple tree leaves in supper layer (A), middle layer (B), and lower layer (C) of canopy. (CK: control group)

treated with S_{3307}, and 84% and 38% treated with DTA-6 higher than that in the control group. After 21, 36, and 46 days treatment, the net photosynthetic rates of the lower layer leaves were increased 45%, 31%, 334%, and 76% treated with S_{3307}, and 44%, 140%, 174%, and 229% treated with DTA-6 comparing that in the control group. The peak value was reached after 36 days of the treatment.

Difference in light response curve of apple tree leaves in different canopy layers

With the increase of light intensity, the photosynthetic rates of the crown surface and middle layer leaves of apple trees first increased, and then, flatten presenting a typical light response columnar feature, which was consistent with the Faquar model (Figure 3). The light saturation points of the crown and middle leaves were 1,072 and 568 μmol/m^2·s, respectively. The photosynthetic rate of leaves on the surface of the canopy was much higher than that in the middle and lower layers with the changes significantly related to light intensity. The leaves on the crown surface of the apple tree were sun leaves, while the leaves in the middle and lower layers were shade leaves.

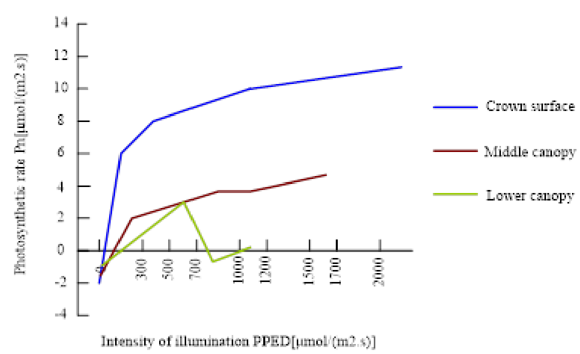

Figure 3. Light response curve of apple tree leaves in different canopy layers.

Photosynthetic characteristics of apple tree leaves in different canopy layers

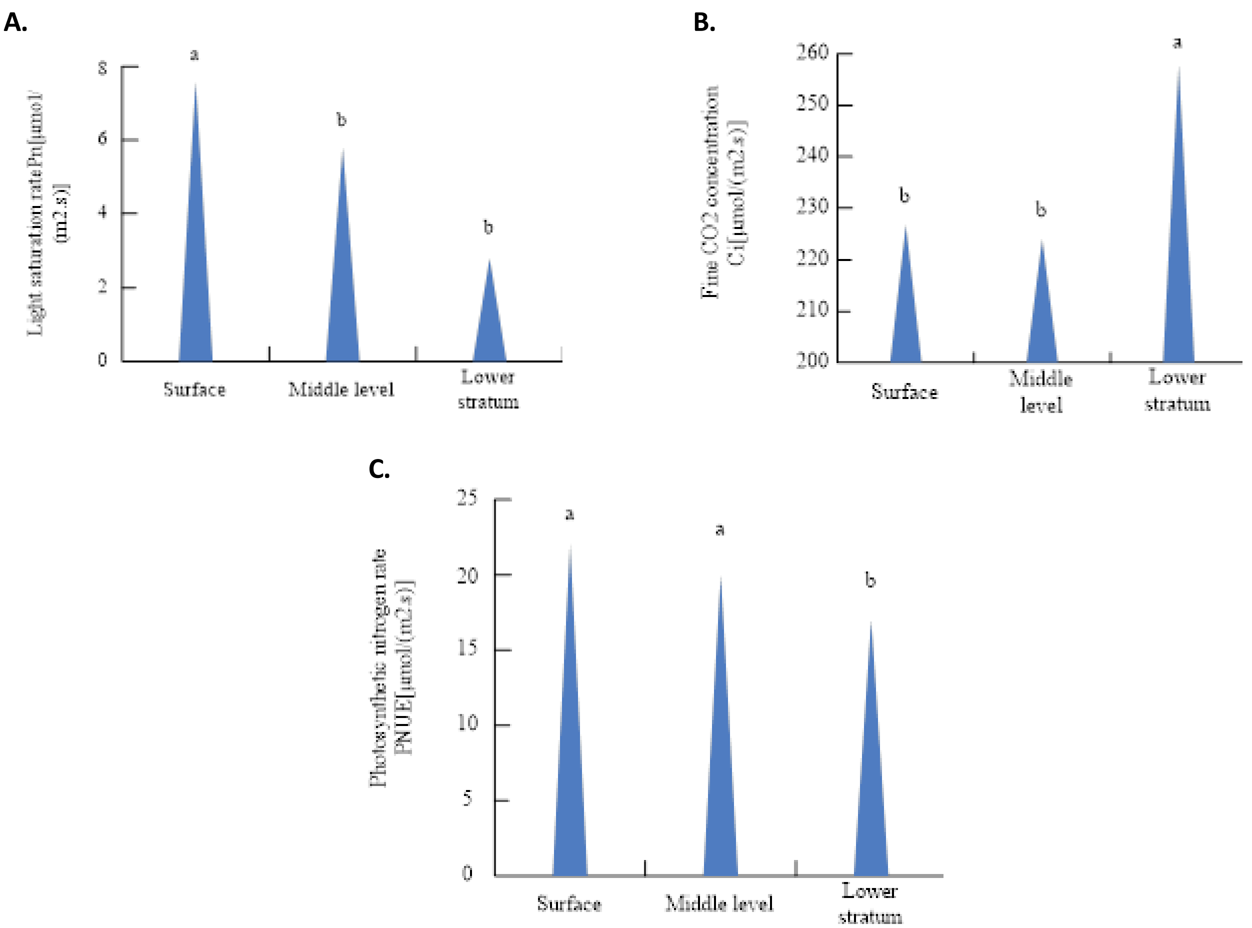

Figure 4. Photosynthesis indexes of apple tree leaves in different canopy. **A.** Light saturation. **B.** Fine CO2 concentration. **C.** Photosynthetic nitrogen rate.

The light saturation rate of apple tree canopy leaves was the highest on the surface followed by the middle layer, and then, the lower layer. The stomatal conductance (Gs) of apple tree leaves decreased in the order of surface layer > middle layer > lower layer. The distribution order of intercellular CO_2 concentration (Ci) of the leaves was lower layer > surface layer > middle layer. The transpiration rate (Tr) decreased in the order of crown surface layer > middle layer > lower layer. The photosynthetic nitrogen efficiency of apple tree crown surface leaves was the highest one, followed by the middle layer, and then, the lower layer (Figure 4).

Relationship between photosynthetic capacity and leaf characteristics of apple trees

Table 1 showed the correlation between photosynthetic ecological factors and physiological factors of apple trees. There was a significant positive correlation between light saturation rate (Pn), the stomatal conductance (Gs), and the transpiration rate (Tr) in leaves, indicating that stomatal changes were closely related to photosynthetic rate. There was a certain degree of negative correlation with intercellular CO_2 concentration (Ci). However, it did not reach a significant level. Correlation analysis showed that Tr was significantly positively correlated with Gs.

Plant photosynthesis is influenced by various factors [14, 15]. With the increase of canopy closure, the photosynthetic active radiation gradually decreases. At the same time, the

Table 1. Correlation of photosynthetic ecological factors and physiological factors of apple tree leaf.

Index	Tr	G_s	C_i	PNUE	N_A	C_A	LMA	Chl_A
P_n	0.93**	0.93**	-0.53	0.88**	0.96**	0.91**	0.92**	0.80**
Tr		0.86**	-0.60	-0.77*	-0.95**	-0.96**	-0.96**	-0.76*
G_s			-0.28	0.82**	0.91**	0.82**	0.83**	0.68*
C_i				-0.56	-0.49	-0.52	-0.53	-0.63
PNUE					0.75*	0.70*	0.71*	0.78*
N_A						0.96**	0.96**	0.76*
C_A							0.99**	0.73*
LMA								0.73*

Note: *$P < 0.05$. **$P < 0.01$.

movement of air and water within the canopy are also altered, which changes the photosynthetic characteristics of leaves in different canopy layers [16]. Many studies have shown that rubisco has a high content and activity in plant leaves under high light intensity, and leaves have strong photosynthetic capacity [17]. Light intensity also directly affects photosynthesis and transpiration [18]. In this study, the photosynthetic active radiation of the lower canopy of apple trees was much lower than that of the crown surface and middle layer leaves. Although Gs decreased significantly with the decreasing of canopy, Ci was the largest in the lower canopy, which indicated that the main factor leading to the decrease in Pn in low canopy leaves was the decrease in photosynthetic active radiation intensity, which further led to a significant decrease in photosynthetic system activity and carboxylation efficiency. The decrease in stomatal conductance might not be the main influencing factor [19].

Due to changes in lighting conditions, the tissue structure of leaves has also changed. Relevant studies have found that changes in canopy light intensity such as changes in leaf cell thickness and protoplast density can affect the photosynthetic potential of plants [20]. This research result also proved this point. The specific leaf weight (LMA) was significantly positively correlated with photosynthetic capacity and light yield. Some researchers used photosynthetic nitrogen efficiency as an effective indicator to evaluate nitrogen participation in photosynthesis. This study showed that under the influence of light intensity, the distribution of carbon, nitrogen, and other elements in apple tree leaves significantly changed, affecting photosynthetic nitrogen efficiency. Although there was a significant difference in the maximum photosynthetic rate between the crown and middle layer leaves, there was no significant difference in photosynthetic nitrogen efficiency. The photosynthetic nitrogen efficiencies of the crown and middle layer leaves were significantly higher than that of the lower layer leaves. There was a significant positive correlation between chlorophyll content and photosynthetic rate (r = 0.806), and also there were significant differences in the ratio of chlorophyll content to total nitrogen in leaves from different canopies. Therefore, how to adopt reasonable cultivation techniques, adjusting the distribution of nitrogen in the canopy, improving nitrogen utilization efficiency, and thereby, improving the photosynthetic capacity of apple tree populations are worth further investigation.

Conclusion

The effects of different canopy layers on photosynthesis characteristic parameters of apple tree leaves were investigated in this study. The results showed that the net photosynthetic rate of the three tree forms increased with the increase of photosynthetic radiation, large canopy surface area, small canopy volume, good ventilation, and strong light intensity, which were the highest in evacuation stratification form, followed by improved spindle, and then, natural round head forms. The chlorophyll content, photosynthetic rate, transpiration rate, and stomatal conductance of leaves in different canopy layers were increased to varying degrees by spraying plant growth regulators, S_{3307} and DTA-6, on apple trees, which enhanced the photosynthesis of leaves. Through the study of photosynthesis characteristic parameters of light saturation rate, stomatal conductance, transpiration rate, chlorophyll content, *etc*., it was found that, in different canopy layers of apple trees, the surface layer had the best photosynthesis effect on apple tree leaves, followed by the middle layer, and then, the lower layer. Therefore, the photosynthetic capacity of apple tree population can be improved by adjusting the canopy leaf distribution of apple trees, so as to rationalize cultivation and increase yield.

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