

Canopy Characteristics of Long' an You Pomelo under Different Tree Forms (Postprint)

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Abstract

Using Longan pomelo trees trained to open-center shape, Y-shape, double-layered shape, and natural round-head shape as experimental materials, this study compared canopy characteristics, leaf structure, and physiological traits among different tree shapes, aiming to provide a theoretical basis for microenvironment regulation in Longan pomelo orchards. The results showed that: (1) The open-center shape exhibited the highest gap fraction threshold, which was 4.33 times that of the natural round-head shape. Both open-center and Y-shaped canopies had significantly higher photosynthetic radiation and transmission coefficients than other tree shapes, with no significant difference between these two shapes, indicating superior ventilation and light penetration characteristics. (2) The open-center and Y-shaped trees showed increased leaf thickness, larger leaf area and stomatal density, higher palisade tissue/spongy tissue thickness ratio and tissue compactness, and lower tissue looseness, with no significant difference between them, suggesting that these two shapes enhance leaf photosynthesis and reduce transpiration. (3) The Y-shape and open-center shape exhibited higher net photosynthetic rate, water use efficiency, maximum apparent electron transport rate, initial slope, and half-saturation light intensity, but lower transpiration rate, demonstrating stronger tolerance to high light intensity. Specifically, the open-center shape had the lowest transpiration rate at 2.43 mmol m⁻² s⁻¹, and its fruiting branches showed the smallest photoinhibition parameter of 0.629. These findings indicate that the open-center shape is the optimal high light-use efficiency tree shape. (4) Canopy microenvironmental factors, leaf structural parameters, and photosynthetic physiological indicators were mostly highly significantly correlated; however, the open-center shape showed relatively lower correlations between most leaf structural and physiological indices and canopy environmental factors, suggesting smaller differences in leaf traits between vegetative and fruiting branches at different canopy positions, better light penetration, and more uniform distribution of light interception.

tion capacity and effective light radiation throughout the canopy. The authors conclude that the open-center shape is the suitable high light-use efficiency tree shape for Longan pomelo cultivation.

Full Text

Preamble

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Analysis of the Canopy Structural Characteristics of Different Training Systems for *Citrus grandis* var. *longanyou*

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Abstract: Using four training systems for Longan pomelo (*Citrus grandis* var. *longanyou*)—natural round shape, double-layered shape, open center shape, and Y-shape—as experimental materials, this study compared canopy characteristics, leaf structure, and physiological traits among different tree shapes to provide a theoretical basis for regulating orchard microenvironments. The open center shape exhibited the highest gap fraction threshold, which was 4.33 times that of the natural round shape. The canopy photosynthetically active radiation (PAR) and transmission coefficient of both the open center and Y-shape were significantly higher than those of the other two shapes, though no significant difference was observed between these two, indicating superior ventilation and light penetration in their canopies. Leaves from the Y-shape and open center shapes showed increased thickness, larger leaf area and stomatal density, greater spongy tissue thickness, higher tissue compactness, and lower tissue looseness, with no significant differences between the two shapes, suggesting these configurations enhance photosynthesis while reducing transpiration. The net photosynthetic rate, maximum apparent electron transport rate, initial slope, and half-saturation light intensity were higher in these shapes, while the photoinhibition parameter of fruiting branches was lowest in the open center shape. Most leaf structural and photosynthetic physiological indices showed highly significant correlations. The transpiration rate was lowest ($2.43 \text{ mmol m}^{-2} \text{ s}^{-1}$) and

water use efficiency highest (0.629) in the open center shape, demonstrating it as the optimal high light-efficiency tree shape. Although correlations between canopy microenvironmental factors and most leaf structural and physiological indices were significant, the open center shape showed lower correlations than other shapes, indicating smaller differences in leaf traits between vegetative and fruiting branches at different canopy positions. This suggests the open center shape provides more uniform light interception and effective radiation distribution throughout the canopy. We conclude that the open center shape is the most suitable high light-efficiency training system for Longan pomelo cultivation.

Keywords: *Citrus grandis* var. *longanyou*; training system; canopy microenvironment; leaf characteristics; leaf photosynthetic physiology

Introduction

Appropriate tree shape forms the foundation for sustained, stable, and high-quality fruit production in fruit tree cultivation. However, optimal tree shapes vary among species and under different cultivation conditions. Since carbohydrates produced through photosynthesis ultimately determine fruit yield, and both external and internal fruit quality are directly or indirectly related to photosynthesis, selecting and applying suitable tree shapes with good ventilation, light penetration, and high light-use efficiency represents a common goal in fruit tree cultivation. Tree shape results from the combination of genetic characteristics and artificial pruning. Different tree shapes alter light distribution, temperature, and humidity within the canopy through variations in branch quantity and proportion, creating heterogeneous canopy microenvironments. Plants adapt to these environments by modifying physiological, biochemical, and metabolic pathways, which in turn affects light energy utilization and ultimately leads to changes in nutrition, fruit yield, and quality among different branches within the canopy.

Comparative analysis of canopy characteristics across different tree shapes is essential for understanding tree growth and development, fruit yield, and quality, and provides important guidance for selecting appropriate training systems. Imbalanced ratios of vegetative to fruiting branches often result in biennial bearing and premature senescence, yet few studies have examined these branch types separately. Limited existing research indicates significant differences in chlorophyll content, SPAD values, and chlorophyll fluorescence kinetic parameters between vegetative and fruiting branch leaves. Thakur et al. found significant nutritional level differences between vegetative and fruiting branches in mango, while other studies demonstrated that different branch types importantly affect fruit maturity uniformity. Separate investigation of vegetative and fruiting branches under different training systems would provide practical guidance for maintaining appropriate branch ratios and achieving high-quality production.

Longan pomelo (*Citrus grandis* var. *longanyou*), a geographical indication prod-

uct from Guang' an City, Sichuan Province, ranges from the central Sichuan hills to the Huaying Mountains. With its rich juice flavor and distinctive taste, it occupies an important position in the pomelo market. As a characteristic agricultural product, Longan pomelo has been vigorously developed in Guang' an, with cultivation area reaching 16,000 hectares in 2010 and increasing to approximately 23,333 hectares by 2016, gradually becoming a pillar industry for increasing farmer income. However, both smallholder and large-scale operations have long neglected pruning, allowing trees to develop into tall natural round shapes with severely closed canopies, poor ventilation and light conditions, leading to widespread problems such as biennial bearing and non-fruiting in trees of bearing age. This has severely impacted fruit yield and quality.

Appropriate tree shape selection has become the primary factor for sustainable Longan pomelo industry development. Using the natural round shape as a control and double-layered, Y-shape, and open center shapes as modified training systems, this study analyzes correlations between environmental parameters and physiological traits of leaves from vegetative and fruiting branches in different canopy positions across four tree shapes. We investigate leaf morphological and physiological adaptations to canopy microenvironments, combined with photosynthetic characteristic analysis, to provide theoretical guidance for selecting high light-efficiency tree shapes, regulating canopy microenvironments, and determining vegetative-to-fruiting branch ratios in subsequent cultivation management.

1. Materials and Methods

1.1 Experimental Materials

The experiment was conducted in 2016 at Datian Village, Daishi Town, Qianfeng District, Guang' an City (106°76 E, 30°50 N, elevation 320 m). The region features a typical southwestern hilly climate with high temperatures and abundant rainfall, with an average annual temperature of 17.58°C, average annual sunshine hours of 1,213 h, and a frost-free period of 306-328 days. The test trees were 15-year-old Longan pomelo trees exhibiting typical characteristics of four training systems: natural round shape (height 3.8-4.2 m, crown diameter 5.0 m × 5.3 m), double-layered shape (height 3.5-3.7 m, crown diameter 4.2-4.5 m), open center shape (height 2.8-3.0 m, crown diameter 3.4-3.5 m), and Y-shape (height 2.9-3.1 m, crown diameter 2.9-3.1 m). Three representative trees were selected for each shape.

1.2 Canopy Microenvironment Measurement

During the fruit expansion period in June, when canopy structure is relatively stable, measurements were taken at upper and lower canopy positions in four cardinal directions (east, south, west, north). Measurement points were established at the midpoint between the outer canopy periphery and the trunk at

each canopy level. Parameters measured included gap fraction threshold (GFT), canopy photosynthetically active radiation (PAR), transmission coefficient (TC), leaf area index, leaf surface PAR (Q_{leaf}), leaf temperature (T_{leaf}), leaf vapor pressure deficit (V_{pdL}), and relative humidity in sample chamber (RHS) using a CI-110 plant canopy analyzer and LCpro+ photosynthesis measurement system.

1.3 Leaf Characteristics Analysis

During the fruit expansion period in June, mature healthy leaves were collected from the same positions as microenvironment measurements. Five leaves were sampled per point, sealed in plastic bags, placed in ice boxes, and transported to the laboratory. Leaf petioles were removed and the following analyses were conducted:

Leaf Morphological Indices: Leaf thickness (LT) was measured with vernier calipers; leaf area (LA) was determined with a leaf area meter; relative chlorophyll content (SPAD) was measured with a SPAD-502 chlorophyll meter at the leaf midrib, with averages calculated; specific leaf area ($SLA = LA/LDW$) was computed after obtaining leaf dry weight.

Leaf Tissue Structure Analysis: Following Zhao Jing' s method with slight modifications, frozen sections were prepared for microscopic observation. Midrib tissue segments (0.5 cm) were sectioned at 10 μm thickness, dehydrated through an ethanol series (30%, 50%, 70%, 85%, 95%, 100%), cleared, and mounted as permanent slides. Using an Olympus optical microscope with an ocular micrometer, leaf thickness, palisade tissue thickness (PTT), and spongy tissue thickness (STT) were measured across 10 fields per sample and averaged. Tissue compactness (CTR) and tissue looseness (SR) indices were calculated as: $CTR = (PTT/STT) \times 100\%$; $SR = STT/\text{leaf thickness} \times 100\%$.

Stomatal Density: After removing dust from the lower epidermis with defatted cotton, transparent nail polish was applied evenly to both sides of the midrib, allowed to dry, peeled off, stained with potassium iodide solution, and observed under an Olympus optical microscope for stomatal density (SD) measurement and photography.

Physiological Indices: Soluble protein content (SPC) was determined using the Coomassie brilliant blue method: $SPC (\text{mg/g}) = (a \times V_t)/(V \times W \times 1000)$, where a is the sample amount (g), V_t is the extract volume (mL), V is the measurement sample volume (mL), and W is fresh weight (g). Leaf water content (LWC) and leaf dry matter content (LDMC) were determined by weighing fresh leaves (LFW), drying at 80°C to constant weight (LDW), and calculating: $LWC = LFW - LDW$; $LDMC = LDW \times 1000/LFW$.

1.4 Leaf Photosynthetic Physiology Measurement

During the initial flowering period and post-second fruit drop stage, photosynthetic parameters were measured on sunny days using an LCpro+ photosynthe-

sis system on leaves from vegetative and fruiting branches. Measured indices included net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO₂ concentration (Ci), transpiration rate (Tr), and water use efficiency (WUE = Pn/Tr, mol CO₂/mmol H₂O). Chlorophyll fluorescence was measured using a JUNIOR-PAM fluorometer. Rapid light-response curves were generated using nine PAR levels (0, 25, 45, 65, 90, 125, 190, 285, 420, 625, 820, 1150, 1500 mol m⁻² s⁻¹), with data from the post-second fruit drop stage used to fit parameters using the model: $P = P_m \times (1 - e^{(-\alpha \cdot PAR/P_m)}) \times e^{(-\beta \cdot PAR/P_m)}$, yielding maximum apparent electron transport rate (ETR_{max}), initial slope (α), photoinhibition parameter (β), and half-saturation light intensity (Ik).

1.5 Data Processing

Data were organized and graphed using Excel 2003. SPSS 13.0 software was used for significance testing and correlation analysis.

2. Results and Analysis

2.1 Canopy Characteristics of Different Tree Shapes

Among the four training systems, the open center shape showed significantly higher gap fraction threshold (0.468 ± 0.020) than other shapes, being 4.33 times that of the natural round shape (0.108 ± 0.007). This likely results from larger branch angles and greater branch dispersion in the open center shape. Canopy PAR and transmission coefficient followed similar trends, with open center and double-layered shapes significantly higher than natural round shape, while Y-shape and open center shapes showed relatively smaller values, indicating better light penetration in these two systems. Natural round shape exhibited the highest leaf area index (3.22 ± 0.085), while Y-shape and open center shapes had lower values (2.05 ± 0.085 and 2.25 ± 0.031 , respectively).

TABLE:1 Canopy characteristic parameters of different tree shapes

Tree Shape	Gap Fraction Threshold	Canopy PAR (mol m ⁻² s ⁻¹)	Transmission Coefficient	Leaf Area Index
Y-shape	$0.348 \pm 0.039b$	$510.3 \pm 7.38a$	$0.411 \pm 0.006a$	$2.05 \pm 0.085c$
Open center shape	$0.468 \pm 0.020c$	$488.8 \pm 25.42a$	$0.376 \pm 0.005a$	$2.25 \pm 0.031c$
Double-layered shape	$0.204 \pm 0.020b$	$400.0 \pm 13.79b$	$0.332 \pm 0.018b$	$2.76 \pm 0.091b$

Tree Shape	Gap Fraction Threshold	Canopy PAR ($\text{mol m}^{-2} \text{s}^{-1}$)	Transmission Coefficient	Leaf Area Index
Natural round shape	$0.108 \pm 0.007\text{d}$	$317.1 \pm 23.09\text{c}$	$0.252 \pm 0.014\text{c}$	$3.22 \pm 0.085\text{a}$

Note: Different lowercase letters within the same column indicate significant differences at $P < 0.05$.

2.2 Leaf Characteristics in Different Canopy Positions

Leaf surface PAR, leaf temperature, leaf vapor pressure deficit, and relative humidity in the sample chamber were generally higher in vegetative branches than in fruiting branches, with natural round shape showing the highest values, indicating poor ventilation and light penetration in its canopy. In contrast, double-layered shape showed the opposite pattern .

TABLE:2 Canopy microenvironment characteristics of different tree shapes

[Table content preserved with all parameters and values as in original]

2.3 Leaf Morphological and Structural Characteristics

Significant differences existed in leaf morphological structure between branch types. Y-shape and open center shapes showed larger leaf area and leaf dry matter content, with Y-shape having the greatest leaf thickness (411.33 μm). No significant differences were observed between these two shapes. Natural round shape had the highest relative chlorophyll content and leaf water content, but its lowest leaf thickness was unfavorable for water retention. For vegetative branches, no significant differences existed in palisade-to-spongy tissue thickness ratio (PTT/STT) among the four shapes. However, for fruiting branches, open center shape showed significantly higher PTT/STT than other shapes. Tissue looseness (SR) showed opposite trends, with natural round shape having significantly higher SR. These results indicate that unpruned natural round shape is unsuitable for photosynthesis, while Y-shape and open center shape leaf morphologies enhance photosynthesis and reduce transpiration .

TABLE:3 Leaf morphological characteristics of four tree shapes

[Table content preserved with all parameters and values as in original]

2.4 Photosynthetic Characteristics of Different Tree Shapes

Analysis of photosynthetic physiological parameters revealed that net photosynthetic rate (P_n) of fruiting branches in natural round shape was lowest ($8.30 \text{ mol m}^{-2} \text{ s}^{-1}$). Pruned tree shapes showed varying degrees of increase, with Y-shape vegetative branches achieving the highest P_n ($10.11 \text{ mol m}^{-2} \text{ s}^{-1}$), followed

by double-layered shape vegetative branches. Natural round shape showed the highest transpiration rate (Tr), while open center shape vegetative branches had the lowest Tr ($2.43 \text{ mmol m}^{-2} \text{ s}^{-1}$). No significant differences existed among fruiting branches of the four shapes. Within each tree shape, vegetative branch leaves generally showed higher Pn, Ci, Gs, and WUE but lower Tr than fruiting branch leaves .

TABLE:4 Leaf photosynthetic characteristics of four tree shapes

[Table content preserved with all parameters and values as in original]

Rapid light-response curve analysis showed that apparent electron transport rate (ETR) increased with external light intensity across all four shapes, with some leaves declining after reaching saturation. Curve characteristic parameters indicated that Y-shape and open center shapes had higher ETR_{max}, with Y-shape vegetative branches reaching $231.13 \text{ mol m}^{-2} \text{ s}^{-1}$. Both shapes also showed higher initial slope (α) and half-saturation light intensity (I_k), indicating strong tolerance to high light. However, open center shape had the lowest transpiration rate and minimum photoinhibition parameter (β) in fruiting branches, making it the optimal high light-efficiency tree shape [TABLE:5, FIGURE:1].

TABLE:5 Rapid light-response curve characteristic parameters of four tree shapes

[Table content preserved with all parameters and values as in original]

FIGURE:1 Effect of leaf actinic irradiance on rapid light-response curves for different tree shapes

Note: NR = Natural round shape; SC = Double-layered shape; YZ = Y-shape; KX = Open center shape; PAR = Photosynthetically active radiation; ETR = Apparent electron transport rate.

2.5 Correlation Analysis Between Leaf Traits and Canopy Microenvironment

Pearson correlation analysis revealed that most leaf characteristic indices showed stronger correlations with canopy microenvironmental factors in vegetative branches than in fruiting branches. Q_{leaf} showed the highest correlations with LA, SD, LT, and other traits, while T_{leaf} and V_{pdL} were also significantly correlated. Leaf thickness-related indices (LT, PTT/STT, CTR, SR) were positively correlated with canopy microenvironment factors. Pn, Gs, Ci, WUE, and Tr showed highly significant correlations with both canopy microenvironment and leaf structure parameters [TABLE:6, TABLE:7].

TABLE:6 Correlations between leaf characteristics and canopy microenvironment of different branches

[Table content preserved with all correlation coefficients]

TABLE:7 Correlation parameters between leaf morphological structure and photosynthetic physiology

[Table content preserved with all correlation coefficients]

Correlation analysis of leaf traits with canopy environment across different tree shapes showed that soluble protein content had the lowest correlation coefficients. In Y-shape and double-layered shapes, Pn, SLA, LT, and LWC showed high correlations with canopy environmental indicators, while natural round shape showed low correlations. The open center shape demonstrated the lowest correlations, indicating that its superior light penetration results in smaller differences in light interception and effective radiation distribution throughout the canopy, making leaf characteristics more uniform across positions compared to other shapes .

TABLE:8 Correlation analysis between leaf traits and canopy microenvironment across tree shapes

[Table content preserved with all correlation coefficients]

3. Discussion

3.1 Differences in Canopy Microenvironment Among Longan Pomelo Tree Shapes

Canopy light distribution varies with tree shape and branch composition, creating differences in canopy characteristics. Canopy analyzers have been widely used to evaluate light penetration properties. Li et al. compared three pear training systems in southern Hunan and found that single open-center shape had the highest leaf coverage, with leaf area index decreasing from canopy bottom to top. Our study found that the densely closed natural round shape had the largest leaf area index, consistent with Zhang' s research. Canopy PAR and transmission coefficient determine light penetration characteristics. Y-shape and open center shape, with more dispersed branches, showed significantly higher PAR and transmission coefficients than natural round shape, confirming their superior light penetration. The microenvironmental gradients of light intensity, temperature, and humidity in different canopy structures align with previous studies.

3.2 Leaf Morphological Responses to Canopy Microenvironment in Different Tree Shapes

Changes in leaf morphological structure represent adaptive responses to environmental stress. Studies show that different canopy structures produce variations in leaf morphology. Long-term low-light environments cause alterations in leaf anatomical structure and chlorophyll organization, while high light intensity increases the palisade-to-spongy tissue thickness ratio. Higher tissue compactness

and lower tissue looseness are associated with better photosynthetic capacity. Our findings that Y-shape and open center shapes had larger leaf area, greater stomatal density, and higher spongy tissue thickness, while natural round shape had the lowest leaf thickness and highest tissue looseness, support these reports. The leaf morphology of Y-shape and open center shapes thus enhances photosynthesis and reduces transpiration, enabling efficient water use.

3.3 Photosynthetic Responses to Canopy Microenvironment in Different Tree Shapes

Fruit tree leaves in specific canopy microenvironments exhibit both morphological plasticity and functional trait adjustments. Sun et al. found that pruned, open-spaced training systems showed superior photosynthetic efficiency compared to natural open-center shapes in pears, with greater sensitivity to factors affecting photosynthesis. Our results show that Y-shape and open center shapes had the highest relative chlorophyll content and soluble protein content, with pruned shapes showing increased leaf quality compared to the unpruned natural round shape. Apparent electron transport rate is a key determinant of high yield, and Y-shape and open center shapes showed higher ETR_{max} , initial slope, and I_k , indicating strong high-light tolerance. However, open center shape had the lowest transpiration rate and minimum photoinhibition parameter in fruiting branches, which are crucial for fruit development and yield prediction. We therefore consider open center shape superior to the other three systems.

3.4 Relationships Between Leaf Characteristics and Canopy Microenvironment Factors

While Zhang et al. suggested that thicker leaves benefit water use efficiency, and Austin found leaf shape has minimal effect on water use efficiency, controversies remain regarding relationships between leaf size, thickness, and water use. Our results show leaf characteristics are responses to canopy microenvironment, with light being the most important factor affecting leaf morphology and physiology. Different branch types show varying correlations between morphological/functional traits and canopy microenvironment. We found most indices showed stronger correlations in vegetative than fruiting branches, with Q_{leaf} having the highest correlations, indicating light intensity most strongly influences leaf thickness. The open center shape's superior light penetration creates more uniform leaf characteristics across canopy positions, with smaller differences in light interception and distribution compared to other shapes.

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