



Article Possibility of Increasing the Growth and Photosynthetic Properties of Precocious Walnut by Grafting

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Abstract: Plant growth characteristics after grafting are mainly dependent on photosynthesis performance, which may be influenced by grafting combinations with different rootstocks and scions. In this study, we used one-year-old walnut grafts to investigate the grafting compatibility between precocious ('Liaoning 1', L) and hybrid ('Zhong Ning Sheng', Z) walnut, as well as rootstock and scion impact on the growth and photosynthetic properties of walnut trees. The results showed that grafting compatibility between the two varieties is high, with survival rates upward of 86%. Overwintering survival of grafted seedlings was as high as 100%, which indicated that the allopolyploid had good resistance to low-temperature stress. The homograft of the hybrid walnut had the highest net photosynthesis rate (18.77 μ mol·m⁻²s⁻¹, Z/Z) and growth characteristics, which could be due to its higher transpiration rate and stomatal conductance, whereas the homograft of precocious walnut presented the lowest net photosynthesis rate (15.08 μ mol·m⁻²s⁻¹, L/L) and growth characteristics. Significant improvements in the net photosynthesis rate (15.97 and 15.24 μ mol·m⁻²s⁻¹ for L/Z and Z/L, respectively) and growth characteristics of precocious walnut were noticed during grafting of the hybrid walnut, which could have been contributed by their transpiration rate. The results of this study serve as a guide for the selection and breeding of good rootstock to improve plant growth characteristics and photosynthetic efficiency. We conclude that good rootstock selection improves plant growth potential and could play an important role in sustainable production.

Keywords: Juglans spp.; grafting; growth characteristics; photosynthesis; sustainable production

1. Introduction

Walnut (*Juglans* spp.) has a wide distribution and is globally planted for its nuts and wood. Due to their oil content and quality, walnuts are known as the "king of plant oils". The fat content in walnut kernels ranges from 60% to 75%, with unsaturated fatty acids accounting for more than 90% of total fat content [1]. The two most widely cultivated species of walnuts for commercial global nut production are the *J. regia* and *J. sigillata*. *J. hindssii* and *J. nigra* are cultivated for commercial nut and wood production in the USA [2]. In recent years, walnut has been favored due to its high nutritional value, and has become widely planted, cultivated, and cropped. According to the statistics of the United Nations Food and Agriculture Organization (FAO), walnut harvest acreage in the world was >1,549,710 hectares, with China leading global production with 1,785,879 tons in 2018, which

equaled 47.65% of the total world production (http://www.fao.org/faostat/en/#data/QC, accessed in June 2020). Because conventional walnut cultivars only begin to bloom within six years of planting and enter the full fruiting period eight years later, they require large investments in the early stages of growth [3]. However, precocious walnut cultivars that can begin to bloom within one year of planting, entering the full fruiting period five years later, were developed by Liu et al. using intraspecific hybridization breeding between Persian walnut (*J. regia*) germplasms '10103' and '11001' [3,4]. As a result, the selection and breeding of precocious varieties has become the main focus of walnut breeding in the last 20 years [3,5–8]. The precocious walnuts ('Liaoning 1') have been widely cultivated and used for their cold and drought tolerance, faster nut production, thin-shelled nuts, and high yield, favored by the majority of growers and consumers in Northern China [4]. However, the extremely early fruiting onset of substantial nuts broke the balance between tree vegetative growth and reproduction [9]. This can result in trees with low uniformity that are susceptible to premature aging, greater susceptibility to pests and diseases, and declining plant growth potential [10,11]; these factors impede sustained high yields and have hindered the sustainable development of the walnut industry.

Plant growth characteristics are mainly affected by both genetic and environmental factors. Increasing plant photosynthesis efficiency can improve these characteristics [12–14]. At present, they are mainly measured through plant breeding and biotechnologies to optimize light and dark reactions, as well as source–sink carbohydrate transport for improving plant photosynthesis [15–20]. Grafting, however, which is one of the most important techniques for improving fruit yield and quality, results in new allopolyploid species [21]. Thus, grafting has been used to explore how rootstock selection can improve plant growth potential. Examples such as pear [22,23], peach [24], apple [25,26], citrus [27], and grape [28,29] have shown that good rootstock significantly improves plant growth and the fruit maturation period. Photosynthesis is the basic physiological process of plant growth that provides the necessary materials and energy. However, few studies have revealed the effect of rootstock on scion photosynthetic properties. Therefore, investigating key factors that affect the photosynthetic characteristics of the scion is essential for cultivating new rootstock varieties and improving plant productivity.

In recent years, attention has increased on the use of interspecific hybrids for rootstock breeding, such as RX1 ("Walnut Rootstock Clones", 2007) and VX211 [30], which are resistant to *Phytophthora* and nematodes. These were developed by Gale et al. from *J. microcarpa* × *J. regia* and *J. hindsii* × *J. regia*, respectively. Rootstock breeding studies have been relatively lagging in China; however, Pei et al. [1] started a distant crossbreeding program using *J. regia*, *J. hindssii*, *J. nigra*, and *J. major* as breeding parents. As a result, hybrid walnut varieties were selected and bred ('Zhong Ning Sheng', *J. hindsii* × *J. regia*) that grow quickly, produce good-quality wood, and are resistant to environmental stress [31]. Rootstock, which comprises the transport channel for nutrients, water, and photosynthetic products, is grafted with a scion to form a symbiont in which the rootstock and scion have both a competitive and a symbiotic relationship with each other [32,33]. We hypothesized that the grafting allopolyploid species (2n = 32) [34] in both the precocious and hybrid walnut likely improved plant growth characteristics, which solves the problem of premature aging of precocious walnut and results in the good-quality wood of hybrid walnut for sustainable production.

In this study, we explored these questions using the square bud grafts of one-year-old plants of precocious ('Liaoning 1', L) and hybrid walnut ('Zhong Ning Sheng', Z) trees. We assessed the performance of the growth and photosynthetic properties of both homo-grafted (L/L, Z/Z) and heterografted (Z/L, L/Z) combinations. We used a structural equation model to explore the keys factors that affected the photosynthetic characteristics of the scion. The results of this study serve as a guide for identifying good rootstocks for grafting and provide information that can be applied in rootstock breeding.

2. Materials and Methods

2.1. Materials and Study Site

The study was conducted on an experiment field in Baoding in the Chinese province of Hebei $(39^{\circ}07' \text{ N}, 115^{\circ}12' \text{ E})$. This region has a temperate monsoon climate with an average annual temperature of 10.7 °C (annual minimum = -20 °C, maximum = 39 °C), and mean annual precipitation in the study area was 545 mm in 2018–2019. The site is characterized by flat terrain, sandy loam soils, and medium management. In May 2018, we took one-year-old trees of both precocious ('Liaoning 1', used for nut production) and hybrid ('Zhong Ning Sheng', used for wood production) walnut for use as rootstocks. Scions were collected from the plants in the local scion garden that showed vigorous and consistent growth. We collected buds from the central portions of new shoots (approximately 0.5–0.8 cm diameter) of both varieties using square bud grafting (LY/T 3004.3-2018) to create two homograft ('Liaoning 1'/'Liaoning 1' or L/L; 'Zhong Ning Sheng'/'Zhong Ning Sheng', or Z/Z) and two heterograft ('Zhong Ning Sheng'/'Liaoning 1', or Z/L; 'Liaoning 1'/'Zhong Ning Sheng', or L/Z) combinations. Each grafting combination treatment consisted of 100 trees. Routine field management of grafted seedlings was conducted after grafting. Grafts were unbound after 25 days, and expansion or germination of the scion bud was used as an indication of successful grafting.

2.2. Graft Compatibility and Plant Characteristics

The compatibility (% survival) of all four rootstock–scion combinations was assessed for the 400 grafted seedlings 25 days after grafting. The overwintering capacity of the grafted seedlings was assessed in the spring of the following year, when plants began to germinate. The new shoots' lengths and branching numbers were measured after 45 days of growth. Plant growth is expressed in terms of the plants' new shoot lengths and branch numbers.

2.3. Leaf Gas Exchange and Chlorophyll Fluorescence Parameter Measurements

The measurements of leaf gas exchange were taken three times in May 2019 on three sunny days between 9:00 a.m. and 5:00 p.m. using a Li-6400XT portable photosynthesis system (LI-6400; Li-Cor, Inc., Lincoln, NE, USA). Measurements were taken every 2 h to characterize daily variation in photosynthesis, using three healthy slices of the parietal lobe from a compound leaf from 10–15 grafted seedlings in each grafting combination. Each treatment had three replicates in each combination. Photosynthetic indicators included net photosynthesis rate (*Pn*), transpiration rate (*Tr*), stomatal conductance (*gs*), intercellular CO₂ concentrations (*Ci*), and water use efficiency (WUE), calculated as WUE = *Pn/Tr*. Photosynthetically active radiation (PAR) and CO₂ concentrations were set at 1200 μ mol·m⁻²s⁻¹ and 400 μ mol·mol⁻¹, respectively. Leaf chamber temperature was set to 25 °C (±0.5 °C), and relative humidity was set to 60% (±5%). Ambient temperature was 24–34 °C during the test. The following night, we subjected the plants to a dark treatment after 30 min and measured the following chlorophyll fluorescence parameters: Minimal (Fo) and maximal (Fm) fluorescence, maximal photochemical efficiency of photosystem II complex (PSII) in the dark (Fv/Fm), potential activity of PSII in the dark (Fv/Fo), and non-photochemical quenching (NPQ). Each seedling was assessed 10–15 times with a FluorPen-FP100 fluorometer (Photon Systems Instrument, Brno, Czech Republic).

2.4. Statistical Analyses

We tested the photosynthesis and chlorophyll fluorescence parameters for differences between grafting combinations using one-way analyses of variance (ANOVAs) in SAS (ver. 9.1, SAS Institute, Cary, NC, USA). The least-significant-difference test was used to evaluate results, and differences were considered significant at p < 0.05. We also developed a structural equation model (SEM) to identify the effects of microenvironmental factors and chlorophyll fluorescence parameters on photosynthetic efficiency to gain a mechanistic understanding of how transpiration rate, stomatal conductance, intercellular CO₂ concentrations, water use efficiency, and chlorophyll fluorescence parameters

(Fo, Fm, Fv/Fo) mediate variation in net photosynthesis rate [35]. In healthy plants, transpiration indirectly reflects the plants' ability to absorb or transport water and nutrients, thus affecting photosynthesis and growth characteristics. Transpiration also affects stomatal opening, CO_2 exchange, and evapotranspiration. Therefore, our model assumed that transpiration directly and indirectly affects net photosynthesis rate via changes in stomatal conductance, intercellular CO_2 concentrations, water, and water potential status by transpiration pull; these factors are thought to be the primary drivers of differences in growth between 'Liaoning 1' and 'Zhong Ning Sheng'. Amos (ver. 22.0, IBM Corporation, Armonk, NY, USA) was used to construct the SEM. All variables were standardized by Z transformation (mean = 0, standard deviation = 1) using the scale function in R software.

3. Results

3.1. Growth Characteristics and Chlorophyll Fluorescence Parameters of Different Grafting Combinations

The growth characteristics and chlorophyll fluorescence parameters of the four grafting combinations are shown in Table 1. Grafts healed well, with survival rates of 86% to 95% (95% for L/L, 88% for L/Z, 90% for Z/Z, and 86% for Z/L). The overwintering survival of grafted seedlings was as high as 100% for some grafting combinations, which indicated that the precocious and hybrid walnut had good compatibility and resistance to low-temperature stress.

Table 1. Plant growth characteristics and chlorophyll fluorescence parameters of different grafting combinations. Data are represented as means (\pm SE); differences between treatment groups were assessed by one-way analysis of variance (ANOVA) in SAS. Means in same column followed by different letters were significantly different according to a least-significant-difference test at *p* < 0.05. L/L, 'Liaoning 1'/'Liaoning 1'/'Liaoning 1'/'Zhong Ning Sheng'; Z/Z, 'Zhong Ning Sheng'/'Zhong Ning Sheng'; Z/L, 'Zhong Ning Sheng'/'Liaoning 1'.

| | Grafting Combination | | | |
|---------------------------------|----------------------|------------------|------------------|------------------|
| | L/L | L/Z | Z/Z | Z/L |
| Healed | good | good | good | good |
| Survival rate (%) | $95 \pm 2.1a$ | 88 ± 3.8bc | 90 ± 1.6ab | $86 \pm 2.4c$ |
| Overwintering survival rate (%) | 100 | 100 | 100 | 100 |
| New shoots' lengths (cm) | 63.37 ± 1.1c | $67.62 \pm 2.2b$ | $76.87 \pm 2.7a$ | $69.16 \pm 1.5b$ |
| Branching numbers (twig) | $11 \pm 2b$ | $12 \pm 2b$ | $16 \pm 2a$ | $15 \pm 2a$ |
| Fo | 14,531a | 14,792a | 12,589c | 13,158b |
| Fm | 67,061a | 67,086a | 62,900b | 65,615a |
| Fv/Fm | 0.791a | 0.799a | 0.800a | 0.801a |
| Fv/Fo | 3.615b | 3.535b | 3.997a | 4.039a |
| NPQ | 0.973a | 1.018a | 1.053a | 0.900a |

Analyses of productivity after 45 days of spring growth indicated that new shoots' lengths and branching numbers differed among the four grafting combinations. New shoots' lengths of L/L, L/Z, Z/Z, and Z/L were 63.37, 67.62, 76.87, and 69.16 cm, respectively. Branching numbers for L/L, L/Z, Z/Z, and Z/L were 11, 12, 16, and 15 twigs, respectively. The grafting combination of 'Zhong Ning Sheng' as rootstock had longer new shoots and higher branching numbers than those of 'Liaoning 1' as rootstock when the scions were the same, which suggested that 'Zhong Ning Sheng' rootstock improved the plant growth characteristics in precocious walnut ('Liaoning 1').

We analyzed the chlorophyll fluorescence parameters of the four grafting combinations in dark-adapted tissue (Table 1). Scion type significantly affected Fo, Fm, and Fv/Fo (p < 0.05, one-way ANOVA), but there were no significant differences between grafting combinations when the same scion was used (p > 0.05), which indicated that the scion was responsible for the observed differences. Higher energy use efficiency was measured in plants where 'Zhong Ning Sheng' was used as the scion, as demonstrated by the significantly higher values of Fv/Fo and low values of Fo for the Z/L and Z/Z

combination (Table 1). Chlorophyll fluorescence parameters ranged from 0.791 to 0.801 for Fv/Fm and from 0.9 to 1.053 for NPQ; differences between grafting combinations were not significant (p > 0.05). These results implied that the four grafting combinations were not under stress (Fv/Fm about 0.8) and had the same excess excitation energy dissipated in the form of heat.

3.2. Photosynthetic Characteristics of Different Grafting Combinations

Net photosynthesis rate (*Pn*) showed a double-peaked curve throughout the day (Figure 1); it increased rapidly after 9:00 a.m., peaked at 11:00 a.m., then declined rapidly, reaching its minimum at 3:00 p.m., after which it began increasing again. Net photosynthesis rate differed significantly (p < 0.05) between grafting combinations. The highest values were measured in Z/Z plants; L/Z and Z/L were equal, and the lowest values were measured in L/L plants at 11:00 a.m. Z/Z and L/Z showed 18.77 and 15.97 µmol·m⁻²s⁻¹, respectively, compared to 15.08 and 15.24 µmol·m⁻²s⁻¹ for L/L and Z/L, respectively. The overall net photosynthesis rate was lower for Z/L than for Z/Z, but L/Z had a higher net photosynthesis rate than L/L during the daytime. Grafts of L/L consistently had the lowest daytime net photosynthesis rate compared to the three other grafting combinations.



Figure 1. Comparison of diurnal changes of net photosynthesis rate in walnut grafted with four kinds of grafting combinations. Data are represented as means (\pm SE); differences between treatment groups were assessed by one-way ANOVA in SAS. Bars with different letters are significantly different according to a least-significant-difference test at *p* < 0.05. L/L, 'Liaoning 1'/'Liaoning 1'; L/Z, 'Liaoning 1'/'Zhong Ning Sheng'; Z/Z, 'Zhong Ning Sheng'; Z/Z, 'Zhong Ning Sheng'; Z/Z, 'Zhong Ning Sheng'.

The grafting combination significantly affected daytime leaf transpiration rate (Figure 2). With the exception of Z/Z grafts, transpiration rate reached its peak at 11:00 a.m. and its minimum at 5:00 p.m. 'Zhong Ning Sheng' rootstocks increased transpiration in 'Liaoning 1' (L/Z) scions, but 'Liaoning 1' (Z/L) rootstocks decreased transpiration in 'Zhong Ning Sheng' (Z/Z). The grafting combination of 'Zhong Ning Sheng' as rootstock had a higher transpiration rate than that of 'Liaoning 1' as rootstock when the scions were the same. This suggested that the hybrid walnut rootstocks had strong transpiration pull with absorption and transport nutrition.



Figure 2. Comparison of diurnal changes of transpiration rate in walnut grafted with four kinds of grafting combinations. Data are represented as mean (\pm SE); differences between treatment groups were assessed by one-way ANOVA in SAS. Bars with different letters are significantly different according to a least-significant-difference test at *p* < 0.05. L/L, 'Liaoning 1'/'Liaoning 1'; L/Z, 'Liaoning 1'/'Zhong Ning Sheng'; Z/Z, 'Zhong Ning

The diurnal variation in stomatal conductance (*gs*) for the four grafting combinations is shown in Figure 3. Stomatal conductance increased rapidly after 9:00 a.m., peaking at 11:00 a.m. for L/Z and L/L and at 1:00 p.m. for Z/Z and Z/L. At this point, stomatal conductance in L/Z, L/L, and Z/L gradually decreased, whereas in Z/Z, it reached its minimum at 3:00 p.m. Stomatal conductance in plants with 'Zhong Ning Sheng' scions (Z/Z and Z/L) decreased sharply between 1:00 and 3:00 p.m. Z/Z plants consistently maintained the highest daytime stomatal conductance of all four groups.



Figure 3. Comparison of diurnal changes of stomatal conductance in walnut grafted with four kinds of grafting combinations. Data are represented as mean (\pm SE); differences between treatment groups were assessed by one-way ANOVA in SAS. Bars with different letters are significantly different according to a least-significant-difference test at *p* < 0.05. L/L, 'Liaoning 1'/'Liaoning 1'; L/Z, 'Liaoning 1'/'Zhong Ning Sheng'; Z/Z, 'Zhong Ning Sheng'; Z/Z, 'Zhong Ning Sheng'; Z/Z, 'Zhong Ning Sheng'; Z/Z, 'Zhong Ning Sheng', Z/Z, 'Zhong Ning Sheng'; Z/Z, 'Zhong Ning Sheng'; Z/Z, 'Zhong Ning Sheng', Z/Z, 'Liaoning 1'.

Photosynthesis significantly reduces intercellular CO₂ concentrations (*Ci*) in leaves (Figure 4). Intercellular CO₂ concentrations in grafted seedlings showed a downward trend prior to 11:00 a.m., at which point they began to gradually increase in L/L, L/Z, and Z/L; concentrations peaked at 1:00 p.m. in Z/L and at 3:00 p.m. in L/L and L/Z. Concentrations began declining again after 3:00 p.m. in all four grafting combinations. The grafting combination significantly affected intercellular CO₂ concentrations in leaves (p < 0.05); intercellular CO₂ concentrations were significantly higher in Z/Z than those in L/L throughout the day. Intercellular CO₂ concentrations were significantly lower in Z/L than in Z/Z, but significantly higher in L/Z than in L/L (p < 0.05).



Figure 4. Comparison of diurnal changes of intercellular CO₂ concentrations in walnut grafted with four kinds of grafting combinations. Data are represented as mean (± SE); differences between treatment groups were assessed by one-way ANOVA in SAS. Bars with different letters are significantly different according to a least-significant-difference test at *p* < 0.05. L/L, 'Liaoning 1'/'Liaoning 1', L/Z, 'Liaoning 1'/'Zhong Ning Sheng'; Z/Z, 'Zhong Ning Sheng'/'Zhong Ning Sheng'; Z/L, 'Zhong Ning Sheng'.

3.3. Water Use Efficiency of Different Grafting Combinations

Water use efficiency (WUE) in plants varied throughout the day (Figure 5). During the test period, it declined slowly after 9:00 a.m., reaching its minimum at 11:00 a.m. at 4.2 and 3.2 mol·mmol⁻¹; L/L reached its minimum of 5.2 mol·mmol⁻¹ at 1:00 p.m. Before 1:00 p.m., it was the lowest in Z/Z between the four grafting combinations. WUE was significantly lower in L/Z than in L/L, but significantly greater in Z/L than in Z/Z (p < 0.05), which indicated that grafting affects leaf WUE.

3.4. Influential Factors on Net Photosynthesis Rate of Grafted Seedling

We constructed an SEM to explore the interactive effects of internal leaf factors and chlorophyll fluorescence on net photosynthesis (Figure 6). The final model proved to be a good fit to the data ($\chi^2 = 1.283$, p = 0.261, goodness-of-fit index (GFI) = 0.978, Akaike information criterion (AIC) = 67.7, root mean square error of approximation (RMSEA) = 0.058), which indicated that our hypothesis was well supported by the data. The model showed that net photosynthesis rate (Pn) was significantly influenced by internal leaf factors and chlorophyll fluorescence. Transpiration rate (Tr) and stomatal conductance (gs) were significantly positively correlated with net photosynthesis rate, with correlation coefficients of 0.877 and 0.735, respectively. However, WUE and minimal fluorescence (Fo) were significantly negatively correlated with net photosynthesis rate, with coefficients of -0.677 and -0.522, respectively. In addition, the model showed that internal leaf factors and chlorophyll fluorescence indirectly influenced net photosynthesis rate, with transpiration rate (Tr).

stomatal conductance, and intercellular CO_2 concentrations significantly positively correlated and stomatal conductance, intercellular CO_2 concentrations, and WUE significantly negatively correlated. Transpiration, Fv/Fo, Fv/Fo, and net photosynthesis were positively correlated.



Figure 5. Comparison of diurnal changes of water use efficiency in walnut grafted with four kinds of grafting combinations. Data are represented as mean (\pm SE); differences between treatment groups were assessed by one-way ANOVA in SAS. Bars with different letters are significantly different according to a least-significant-difference test at *p* < 0.05. L/L, 'Liaoning 1'/'Liaoning 1'; L/Z, 'Liaoning 1'/'Zhong Ning Sheng'; Z/Z, 'Zhong Ning Sheng', Z/Z, 'Zhong Ning Sheng'; Z/Z, 'Zhong Ning Sheng', Z/Z, 'Zhong Ning Sheng'; Z/Z, 'Zhong Ni



Figure 6. Effects of internal leaf factors and chlorophyll fluorescence parameters on net photosynthesis rate. The low Chi-squared (χ^2), nonsignificant probability level (p > 0.05), high goodness-of-fit index (GFI > 0.90), low Akaike information criteria (AIC), and low root mean square error of approximation (RMSEA < 0.05) below the structural equation models (SEMs) indicated that our data matched the hypothetical models. Green and brown arrows indicate positive and negative relationships, respectively. * p < 0.05 and ** p < 0.01. Solid and dotted lines represent significant and insignificant differences, respectively; r^2 values indicate proportion of variance explained for each variable.

4. Discussion

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Photosynthesis plays a crucial role in plant growth characteristics [36,37]. Synthetic and molecular biology techniques are the most widely used biotechnologies for increasing the efficiency of photosynthesis [38,39]; however, in recent years, grafting has been increasingly used in economically important tree species [22–27]. In this study, the allopolyploid formed by grafting with precocious and hybrid walnuts had good grafting compatibility; survival rate was slightly higher for homo- than hetero-graft combinations, but it was high (>86%) for all combinations (Table 1). This indicated that square bud grafting, like other grafting methods (i.e., side stub, omega, chip budding, and whip tongue), promotes a high survival rate and normal growth in grafted walnut trees [40–43]. Failure to survive (<14%) after healing may be attributable to a failure to protect bud meat during grafting [44]. Our results demonstrated that different rootstock and scion combinations have different and often significant effects on tree growth and photosynthesis rate and growth characteristics of 'Liaoning 1' (L/Z > L/L), but using 'Liaoning 1' as rootstock decreased the net photosynthesis rate of 'Zhong Ning Sheng' (Z/L < Z/Z), which indicated that differences in plant growth characteristics can be attributed to the interaction of the rootstock and scion; this conclusion is consistent with that of Rezaee et al. [45].

Previous studies showed that external environmental factors (i.e., light, temperature, water, O_2 , and CO₂) form the basis of photosynthesis, whereas internal leaf factors (i.e., chlorophyll, leaf area index, stoma) are factors limiting photosynthesis [46–48]. Decreasing antenna size [13,15] and optimizing plant architecture [14,49] are proven means of improving photosynthetic efficiency. However, higher stomatal conductance leads to high transpiration, and high photosynthesis was observed in a previous study [50], which reported results differing from ours. In this study, we found that stomatal conductance in Z/Z and Z/L peaked at 1:00 p.m., but net photosynthesis and transpiration showed a downward trend at 1:00 p.m., but peaked at 11:00 a.m., which indicated that stomatal conductance was not the key factor in increasing net photosynthesis rate within a certain range. The significant difference in net photosynthesis rates could have mainly resulted from their different transpiration behaviors (Figure 2). The Z/Z combination with the highest transpiration had the highest net photosynthesis rate. The L/L combination with the lowest transpiration rate had the lowest net photosynthesis rate. The net photosynthesis rate of the scion with different rootstock combinations was significantly correlated with transpiration capacity, indicating that the difference in net photosynthesis rate could have resulted from the different transpiration behaviors of the L/L, L/Z, Z/Z, and Z/L combinations. As mentioned above, 'Zhong Ning Sheng' as rootstock significantly impacted net photosynthesis rate and growth characteristics in 'Liaoning 1' as scions; this may have resulted from the effects of the rootstock on transpiration in the scion (Figure 2), as transpiration pull promotes absorption and transportation in trees, which results in better plant growth characteristics [51,52]. We found that net photosynthesis rate showed a double-peaked curve, with the first peak at 11:00 a.m. (Figure 1), followed by a reduction in both net photosynthesis and transpiration rate between 11:00 a.m. and 3:00 p.m. It is possible that the higher air temperatures and vapor pressure deficit at noon resulted in stomatal closure [53–55], whereas the walnut root is fleshy and without root hairs, so higher soil temperatures might have influenced absorption and transportation in roots, which significantly reduces transpiration, thereby also reducing net photosynthesis rate [56,57]. Although net photosynthesis rate increased and reached a second peak at about 5:00 p.m., this was lower than the first. This is likely because non-structural carbon that accumulates in leaves suppresses net photosynthesis rate [58,59].

All grafting combinations with different rootstocks and scions provided excellent photosynthesis for plant growth characteristics despite their different internal leaf factors. To further explore the effect relationship between gas exchange variables, an SEM was used to evaluate the relationships between net photosynthesis rate and internal leaf factors. The results of this study demonstrated that stomatal conductance and intercellular CO_2 concentrations can be improved by transpiration rate (Figure 6); higher transpiration rate leads to high stomatal conductance and net photosynthesis rate, but also leads to a high influx of CO_2 . However, the results in Figures 2 and 4 indicate that intercellular CO_2 concentrations decreased with increased transpiration rate before 11:00 a.m. Photosynthesis was used for the calculation of WUE; so, the positive relationship could be observed between net photosynthesis rate and WUE, which differed from the SEM results. This may have occurred due to the increased transpiration rate and stomatal conductance that improve net photosynthesis rate, thus increasing intercellular CO₂ use and evapotranspiration capacity [60,61]. Our results showed that the reduced minimal fluorescence (Fo) and enhanced potential activity of PSII in the dark (Fv/Fo) resulted in higher net photosynthesis rate [62]. This is consistent with the results of Li et al. [63], who found that increased Fo in soybean seedlings decreased net photosynthesis rate. These results could be helpful in understanding the mechanisms behind improved plant growth characteristics and photosynthesis in allopolyploid varieties. We explored plant growth and photosynthetic characteristics in hybrid ('Zhong Ning Sheng') and precocious ('Liaoning 1') walnut; however, the growth of the allopolyploid was also affected by the absorption, transport, and assimilation functions of the rootstock, which warrant further investigation.

5. Conclusions

All graft combinations with L/L, L/Z, Z/Z, and Z/L seedlings healed with survival rates upward of 86%. The Z/Z seedling showed the highest net photosynthesis rate and longest new shoot lengths, whereas the L/L seedling had the lowest net photosynthesis rate and shortest new shoots. After grafting, the hybrid walnut ('Zhong Ning Sheng') as rootstock produced positive effects for precocious walnut ('Liaoning 1'), improving plant growth characteristics and increasing net photosynthesis rate. The positive effects of the hybrid walnut rootstock could have been contributed by their transpiration rate and the positive correlations between net photosynthesis rate and stomatal conductance. Our results indicated that the premature aging of precocious walnut could be improved by grafting using the hybrid walnut for sustainable production.

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Abbreviations

(Pn)—net photosynthesis rate; (Tr)—transpiration rate; (gs)—stomatal conductance; (Ci)—intercellular CO₂ concentrations; (WUE)—water use efficiency; (PAR)—photosynthetically active radiation; (Fo)—minimum fluorescence; (Fm)—maximum fluorescence; (Fv/Fm)—maximum photochemical efficiency of PSII; (Fv/Fo)—potential activity of PSII; (NPQ)—non-photochemical quenching; (L)—'Liaoning 1'; (Z)—'Zhong Ning Sheng'.

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